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Ralph M. Combs
Gulf Coast Research Laboratory

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EMBRYOGENESIS, HISTOLOGY AND ORGANOLOGY OF THE OVARY OF *BREVOORTIA PATRONUS*

By

Ralph M. Combs

Gulf Coast Research Laboratory
Ocean Springs, Mississippi

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ABSTRACT

One hundred ninety-one large scale menhaden, *Brevoortia patronus,* ranging in age from larvae to sexually mature females were used in this study. Collections were made in the littoral and shallow off shore waters of the Gulf of Mexico from Dauphin Island, Alabama, westward to West Bay, Louisiana, at intervals throughout a five year period. Using standard paraffin techniques a number of staining methods were employed. Cytological and histological accounts are presented of the tissue elements of the gonads beginning with inception in the larvae, sexual differentiation to the sexes, and the cyclic changes associated with oogenesis and spawning in the mature fish. The microscopic developments occurring during these periods are correlated with gross features of the organ, the ages of specimens, and with seasonal periods. An account of the morphology and physiology of atretic oocytes and ovulated follicles is presented. Using the ovarian components as an index, the time and duration of the spawning season is established as occurring from late October to February or early March with some variance due to environmental factors. From the study it is possible to postulate that this species exhibits intermittent total spawning in the Gulf.

I. INTRODUCTION

This study was originally initiated for the purpose of correlating the cyclic histological changes in the ovaries of mature females of *Brevoortia patronus* with the temporal and spatial aspects of spawning. During its progress, the scope of the investigations was expanded to include the changes involved from the time of their origin to the stage where sex differentiation was complete.

From the time of His (1873), Semper (1875), and Balfour (1878), considerable literature has accumulated on the gonads of fish. These include a number of investigations of the histologic cycles of the ovaries of several of the Clupeidae. Particularly significant is the account provided by Naumov (1956) of oogenesis and the sex cycle of mature females of the Murmansk herring, *Clupea h. harengus.* As might be anticipated from the taxonomic relationship of this species and *B. patronus,* there exists a considerable degree of similarity in the oogenetic processes. Yamamoto (1958) has contributed a description of the manner of yolk formation in *Clupea pallasi* including an account of its cytochemistry. His conclusions regarding vitellogenesis coincide closely with those reached by the author in the case of the large scale Gulf menhaden. Studies by Suttkus and Sundararaj (1961) on *B. patronus* and by Nagasaki (1958) on the Pacific herring, *Clupea pallasi* involved analyses of the fecundity of the species. No reference was made to the oogenetic activities. Clark (1934) published a brief description of the growth stages of the intra-ovarian in the California sardine *Sardina caerulea* utilizing diameter differences of the oocytes rather than cytological detail to establish their degrees of maturity. He also provided an excellent summary from the literature of the scales or criteria relating to ova size which had been employed by various investigators to evaluate degrees of maturity. A more extensive review of the literature pertaining to the maturity

stages of intra-ovarian development of fish ova was published by Naumov (1956).

Nothing has been published on the origin and histogenesis of menhaden gonads. The interest which investigators have given to the larvae of this species has been directed to their number and external morphogenesis. Reintjes (1962) has given a description of cleavage and external changes which occur in the yolk-sac larvae of *B. tyrannus* and *B. smithi* in the Atlantic between North Carolina and Florida. In an attempt to determine the time of spawning and to suggest the locale of its occurrence, Suttkus (1956) analyzed data obtained from collections of *B. patronus* larvae. A relationship was found between larval development and their inshore movement. A somewhat similar approach using larvae to indicate the reproductive activities of *B. patronus* was carried out by Arnold (1958) in the Galveston, Texas area.

II. MATERIALS AND METHODS

A total of 191 larval, post-larval, immature, and mature fish collected during the period from 1959 through 1960 were utilized in this study. The author is indebted to Mr. J. Y. Christmas, Gulf Coast Research Laboratory, Ocean Springs, Mississippi, for his assistance in collecting most of the material, determining fork lengths, and treating it with killing and fixing agents.

Collections were made from West Bay, Louisiana (89°24′ W longitude, 29°32′ N latitude), eastward to Mobile Bay, Alabama (86° W longitude, 31°32′ N latitude). Relevant data pertaining to the specific areas from which samples were taken is recorded in Table I.

It will be noticed from the stations given in the table that in most instances the fish were taken from euryhaline habitats receiving discharges from the Mississippi, Pearl, and Pascagoula Rivers and many small streams as well as from the Biloxi and Mobile Bays. Salinities varied according to the seasons, tides and continental run-off from from about 2 to 25 o/oo in the shoreward waters of the Mississippi and its inlets to approximately 30 to 35 o/oo at stations in the passes between islands and the continental shelf beyond.

In all cases fork length measurements were made while the fish were in a fresh state. Ovarian measurements were made after the gonad was excised, killed and fixed except in the larval and post-larval material, which by reason of the extreme smallness or absence of an organized gonad did not lend itself to a method of direct measurement. In these cases dimensions were computed from serially sectioned material. In view of the prior treatment of the organs with killing and fixing agents, the measurements thus obtained are slightly less than would have been secured from living specimens.

The excised gonads of the larger fish were fixed in Bouin's solution. In the remaining cases fixation was by 10% formalin, except in a few cases where gonads were removed from intact specimens which had previously been preserved or frozen. Larvae and post-larvae were fixed by transecting the body at points immediately an-

TABLE I

Showing by months the numbers, sizes, and areas from which collections were made. Seven collections were made in 1959 during the period from February to December. In 1960 twenty-five collections were made from January to December.

Month	No. Fish	Fork Length (mm)	Area
February	1	188	Sand Island Light, Mobile Bay, Ala.
May	4	201-217	Sand Point, La.
June	1	198	Cat Island, Miss.
August	7	225-237	Chandeleur Island, La.
December	31	164-226	Horn Island, Biloxi, Miss.
December	1	176	S. E. Chandeleur Light, La.
December	11	187-225	Horn Island, Miss.
January	14	144-192	Fort Bayou, Ocean Springs, Miss.[1/]
January	1	165	Dog Keys Pass, Miss.
March	1	207	Gulf Coast Research Laboratory[1/ 2/]
April	9	78-177	Gulf Coast Research Laboratory[1/ 2/]
April	1	222	Biloxi Bay, Miss.
April	2	17- 32	Gulf Coast Research Laboratory[1/ 2/]
May	9	18- 38	Gulf Coast Research Laboratory[1/ 2/]
May	5	189-194	Empire Bar, La.
May	4	144-204	West Bay, La.
May	1	186	Gulf Coast Research Laboratory[1/ 2/]
May	11	23-338	Gulf Coast Research Laboratory[1/ 2/]
June	3	47- 89	Gulf Coast Research Laboratory[1/ 2/]
June	3	80- 86	Gulf Coast Research Laboratory[1/ 2/]
June	9	102-143	Biloxi Bay, Miss.[1/]
August	14	64-113	Gulf Coast Research Laboratory[1/ 2/]
August	5	63- 70	Gulf Coast Research Laboratory[1/ 2/]
August	7	109-141	Belle Fontaine Beach, Ocean Springs, Miss.[1/]
August	2	93-141	Biloxi Bay, Miss.[1/]
August	9	91-166	Biloxi Bay, Miss.[1/]
October	1	131	Belle Fontaine Beach, Ocean Springs, Miss.[1/]
October	5	159-200	Pascagoula Bay, Miss.[1/]
October	3	160-199	Mobile Bay, Ala.[1/]
November	3	116-170	Biloxi Bay, Miss.[1/]
December	11	82-125	Dog Keys Pass, Miss.[1/]
December	2	181-197	27 miles East of North Pass of Miss. River[1/]
Total	191		No. Collections — 32

[1/]—Waters of Mississippi Sound or tributaries thereof.

[2/]—Harbor of Gulf Coast Research Laboratory, Ocean Springs, Miss.

terior and posterior to the organ and subjecting the entire segment to formalin.

Paraffin embedding was employed throughout. Since the gonads in larvae and post-larvae were too undeveloped to handle if excised, the entire segment of the body prepared as mentioned above was serially sectioned. In older fish the ovaries were either embedded entire or if they were too large to handle in this manner, a transverse segment from their mid region was utilized. Cross sections were made of all gonads, and in addition sagittal sections were prepared from a representative number of the organs from juvenile and mature fish. Sections were cut at 10 microns.

All cross sections from the excised organs from juvenile and mature stages were stained with Heidenhain's iron hematoxylin and eosin. Of the excised ovaries selected for the preparation of sagittal sections, one section each was stained by Heidenhain's, Cajal's trichrome connective tissue, and Flemming's safranin-gentian violet-orange G techniques, respectively. The slides derived from each serially sectioned larva and post-larva were alternately stained by the three designated methods.

Ocular grids were used in making oocyte counts.

III. ORIGIN AND EARLY DIFFERENTIATION OF THE GONAD

Gonadogenesis is initiated only after the recently hatched fish have entered estuarine waters. The origin and early differentiation of the elements of the gonad takes place in menhaden having a fork length of between 17 and 70 mm. During the initial part of this growth period, i. e. from 17 to 50 mm, they are embryonically in the larval state although Reintjes (1961) considers as larvae only fish having a length of from 4 to 22 mm from the snout to the end of the vertebral column. Suttkus (1956) considers them as being in the larval stage during the period when their total body lengths are between 20 and 30 mm. The basis for considering fish up to 50 mm as larvae will be given later. It is during this period that the initial events of embryogenesis of the gonad occur including the incorporation and integration of the primordial cellular elements. The subsequent post-larval period is characterized by the differentiation and organization of the cellular components into the definitive arrangement of the immature just before the onset of oogenesis.

(1) Inception and Early Organization of Larval Gonads (17 to 29 mm Fish)

The form of the future organ is not discernible in fish of 17-18 mm (Plate I, Fig. 1). At this period the area of the parietal mesothelium lying at the upper right and left quadrants of the coelom, which is destined to give rise to the germinal epithelium of the organ, portrays a morphology comparable to the outlying areas. At the loci where the gonads will appear, it is constituted of the same low type of simple cuboidal epithelium that occurs throughout this coelomic surface. In the figure it lies to the left of the dark chromatophores as an indistinct thin gray investment. Its indistinctness is due to the pronounc-

ed chromophobia of its cells. The coelom is the lighter area occurring between the mesothelium and the wall of the gut. The superficial cells thus appear contiguous with the underlying irregular layer constituted of numerous melanophores between which pass a few weakly developed fibrous connective tissue elements. Retro-peritoneally to the pigment cells there is a noticeable paucity of cellular and fibrous elements which diminish to an even greater extent in areas beyond the region of incipient gonad formation. No sharp delineation exists between the two regions. Because of the absence of a sharp transition between the aggregation of cells that will become involved in gonad formation and the more distal elements, the exact limits of the germinal region is difficult to determine. As near as could be determined, it occupies an area of about 15 to 19 microns along a body wall transect while its linear extent is approximately 65 microns. The components constituting this retro-peritoneal contribution to the future gonads includes occasional rather delicate connective tissue fibers having a somewhat areolar arrangement, a moderate number of small mesenchymal or fibroblast cells, and a very few primordial germ cells.

In these larvae the primordial germ cells, although confined to the gonadogenic area, are usually few in number and are relatively widely separated from each other. At this period they typically occupy a position nearby or in contact with the melanophores. The proximity of three, possibly four, of the cells (the larger spheroid elements) to the melanophores is shown in Plate I, Fig. 1. Due to the irregular spacing of the pigment cells, there exists between them occasional interstices through which the fibers and cells of the outlying connective tissue pass into the area immediately contiguous with the bases of the presumptive germinal epithelium. Also situated in such crevices an occasional primordial germ cell can be observed, apparently in the process of migration toward the potential germinal epithelium.

The cytological aspects of the primordial germ cells in general conform to the descriptions given by numerous authors for various vertebrates. The somewhat spherical oxyphilic cytosome is relatively large (7 to 9 microns) and displays an absence of formed elements or specific limiting membrane. At its approximate center the prominent spherical nuclear body (mean diameter 4 microns) encloses a moderate or minimal quantity of fine chromatin material dispersed uniformly throughout. Nucleoli are not visible. The karyotheca is quite distinct. Since younger material was not available for study, the origin of the sex cells was not elucidated and their migrations, if any, could not be traced. They have been described as arising in fish from the lateral (intermediate?) mesoderm *(in partis)* by Balfour (1878) and Rabl (1896), *in toto* from intermediate mesoderm by MacLeod (1881), or in the splanchnic mesoderm of the gut wall or its mesentery (Woods, 1902, and Moore, 1937). An insight as to their origin in the Atlantic menhaden, *B. tyrannus*, was not furnished by Kuntz and Radcliffe (1918) in their study of its embryology and early development. Regardless of the point of origin of these cells, it was found in the present study that only about 9 to 15 of them are found in the body wall immediately prior to their migration into the presumptive gonad.

The mesenchymal or fibroblast elements which are destined to be

TABLE II

Data Relating to Size of Gonads for Fish Between 17 mm and 70 mm Fork Length.

Fork Length (1)	Date (2)	Gonad Dimensions (mm) Length (3)	Vertical (4)	Transverse (5)
17 mm	April	1/	1/	1/
18	April	1/	1/	1/
23	April	0.99	.001	.040
24	May	0.97	.002	.039
24	May	1.29	.008	.054
24	May	1.26	.006	.050
29	May	1.60	.091	.039
29	May	1.80	.050	.028
32	April	1.27	.126	.036
32	May	1.31	.105	..039
34	May	1.22	.112	.042
47	June	1.92	.250	.078
61	June	3.10	.290	.090
63	August	2.20	.300	.133
64	August	3.50	.300	.141
66	August	3.40	.300	.127
68	August	3.80	.800	.161
70	August	6.00	.900	.132

1/—Gonadal anlage in too primitive a stage to permit measurement because marginal limits are not distinctly defined.

incorporated in the gonad are derived from either the intermediate or lateral mesoderm cells of the body wall. Prior to their migration into the organ they are cytologically similar to other fibroblasts in this area which are destined to remain in the body wall. A 10 micron transverse section shows from 15 to 25 of these cells loosely aggregated so as to form a poorly organized cord extending anteriorly-posteriorly close to the coelomic epithelium which will shortly thicken to create a germinal ridge. A gradual transition occurs between these condensed fibroblasts and those which extend out into the body wall. Their size, i. e. 3 microns, is about one half the diameter of the sex cells with which they are integrated. Their shape varies from spherical to stelliform, triangular, or rectangular. Each is constituted of a relatively small amount of homogeneous oxyphilic cytoplasm. Their open face nuclei are generally oval and are composed of a considerable quantity of fine chromophobic material enclosed by a delicate moderately basophilic membrane.

When the larvae attain a length of 23-24 mm, the initial morphogenesis of the gonads becomes evident. This is marked by the appearance of a barely perceptible thickening of the presumptive germinal epithelium which produces a slight substention into the dorsal-lateral regions of the coelom (Plate I, Fig. 2). In the illustration, it appears to the left of the dark melanophores as a gray thickening containing a row of black nuclei. Although this thickening is in the form of an anterior-posterior ridge, its exact dimensions can only be approximated since along its marginal areas there occurs a gradual transition between the somewhat cuboidal cells of the ridge and those of the adjacent peritoneum. That gonadogenesis is initiated and proceeds at a greater rate at the head of the organ is indicated by the greater degree of coelomic distention which occurs at that point. As shown in Table II, the length of this anlage in fish of the age being considered varies from 0.97 to 1.29 mm with a mean length of 1.13 mm. Posteriorly it extends to within approximately 1.4 mm of the anterior margin of the anus. The maximum thickness of the epithelial cells constituting the ridge is in the order of 5 microns as related to 0.5 to 1.0 microns for the outlying parietal epithelium of the coelom. The increased thickness of the layer in the germinal region is due in part to a stratification of the earlier single layer into two imperfectly oriented layers and in part to a differentiation of the original low cuboidal elements into higher cuboidal forms.

Not until slightly later does the germinal epithelium of the ridge undergo sufficient coelomic distention to permit the entrance of the primitive germ cells together with some of all of the associated connective tissue elements. As in the case of younger larvae, occasional primitive sex cells together with mesenchymal and fibroblast elements are found to lie more or less contiguous to the base of the layer of germinal ridge cells. While the number and cytology of the germ cells show no apparent change from the condition existing in younger fish, the fibroblastic elements have become somewhat more abundant. Whereas the presumptive gonadal connective tissue cells in the younger larvae evidence a moderate dispersion in the environs of the forming gonad, in the 24 mm fish they are more densely aggregated in a mass which is coextensive with the lateral margins of the germinal ridge. This cellular condensation can be seen in Fig. 2 in which

the fibroblasts are shown as the numerous dark entities. Mitotic activity could not be established in either the sex cells or the fibroblastic components. The increase which appears to occur in the latter element may be accounted for in part as a continuance of a minimal degree of migration of these cells from outlying areas of the body wall mesoderm into the zone of tissue organization. Reduplication of the primordial germ cells apparently does not occur at this period, or if so at least to no significant degree as substantiated by the constance of the number present.

Specific morphogenesis of the gonad becomes evident in larvae having a length of 24 to 29 mm. During this period, it becomes more and more subtended into the coelom first by a broad base which progressively becomes relatively narrower as it gives rise to the primitive mesentery. During this interval, the primitive germ cells and many of the associated fibroblasts pass through the interstices between the melanophores so as to enter the early gonad. The pigment cells are unaffected by these movements, and no selectivity is observed in the sequence by which the germ cells and fibroblasts enter the protruding gonad.

When the larvae reach a length of 29 mm the reproductive organs are solidly packed with cells and are distinctly subtended into the coelom from the dorsal-lateral quadrants by a well established mesentery. This condition is illustrated in Plate I, Fig. 3. Their three dimensional conformation is suggestive of a pea pod or canoe in that they are most robust in the mid two-thirds of their length from which they gradually taper to the anterior and posterior terminations. The degree to which the organ subtends into the coelom also decreases progressively as its ends are approached. In cross section profile at the mid region, the gonadal vertical axis has progressively increased from the condition in 24 mm fish so that now it is about twice as great as the transverse axis, c. f. Table II. Also at this age, the maximum transverse diameter of the organ, unlike adolescent and mature gonads, occurs about midway between its dorsal mesenteric attachment and its lower free margin. In the older gonads the widest transverse extent is in the upper region of the organ. However, as the ends of the structure are approached in the larval state, the maximum width progressively shifts to a more dorsal position until at its terminations the mesentery represents its greatest lateral axis so that in cross section it assumes a V-shape, the apex of which projects towards the coelom.

Comparison with gonads of 24 mm fish reveals that there has occurred during this interval a mean length growth of 0.53 mm, an increment of 0.065 mm along the vertical axis, but little or no change transversely. In the sample studied the transverse extent was actually 0.015 mm less than in the previous age.

Larval forms of this age present a provisional intra-gonadal organization of the cell types derived from the retroperitoneal complex and the epithelium of the germinal ridge. The various elements now become organized in the establishment of the primitive organ (Plate I Fig. 3) which is characteristically solid due to the massive crowding of the cells, although a few minute irregularly shaped cavities or sinusoidal spaces of variable size are present. The presence in them

of a few hemal elements suggests that they are provisional vascular channels. The manner in which these fissures are randomly disposed implies that vascularization involves a tortuous extension of the dorsally situated gonadal artery and veins into the more peripheral areas of the organ. It is not possible to identify a specific endothelial investment of the vascular components.

The marginal epithelium investing the gonads is not definitely delineated from the internal stromal cells. It consists of a low cuboidal form of cell which in some areas transcends to a squamous state. This change in the epithelium from the double layered pronounced cuboidal form of the 24 mm larvae is the result of a rapid stretching of the layer attributable to the sudden entrance of the retroperitoneal elements. The typically flattened vesicular nuclei are distributed with remarkable spatial irregularity and also exhibit considerable variation in their apical-basal positions in the cells. Cell boundaries or basement membranes cannot be perceived. The moderately oxyphilic cytosomes are homogeneously, finely granular.

The stromal elements constitute the principal part of the internal tissues of the organs. Although many of the great numbers of these elements present are derived from the fibroblastic or mesenchymal cell type which was originally present in the retroperitoneal area adjacent to the gonad and which migrated into the gonad with the sex cells, other cells of the stroma are believed to have been derived from the epithelium of the germinal ridge in view of the marked cytological similarity which exists in the transition between the cells of the epithelial investment and the contiguous stromal cells. Gradually, as the deeper parts of the organ are approached, the cells manifest so many morphological modifications that the transition from the more peripheral cell types to those present internally becomes less perceptible. The stromal elements do not reveal cell boundaries, and because of their compactness an amorphous intercellular matrix appears to be absent. Using the form of the prominent vesicular nuclei as a criterion of cell morphology, the stromal or fibroblast cells appear to exist as spheres, short or elongated ovals, or spindle shaped. Some degree or organization occurs with respect to the distribution of these types in that the spindle shaped cells are more intimately associated with the gonia and are therefore referred to as capsule cells. The remaining stromal cells, representing the greatest number, tend to indiscriminately occupy the interstitial areas. Since many transitional forms are present in those areas where stromal cells lie near the outer extent of the capsule cells, a distinct departmentalization of the units does not exist. In such locations the more distal, spherical or oval form of stromal cell progressively changes to elongated, spindle-like forms, and as the gonia are approached they develop a crescentric curvature conforming with the margin of the enclosed germ cell. This transition is first noticeable at a distance of about 8 microns from the margin of each germ cell. Two to four layers of these cells normally constitute the gonial capsule. The length of the cells of the innermost layer is such that only two to three of the cells are necessary to completely circumscribe each of the prominent germinal elements.

Connective tissue fibers in the gonad are either absent or so delicate they cannot be detected with the staining methods used, includ-

ing Cajal's connective tissue stain. Their existence is suggested, however, by the appearance of an indistinct pattern of undulations in the stromal substance, particularly in the environs of the germ cells. It is possible, however, that this is an artifact caused by a condensation of other materials at the cell boundaries of the capsule cells.

Considerable deviation occurs in the number of gonia present in various segments of the organ. Their frequency varies between 0.8 and 1.7 cells per 100 microns linear distance near the mid-area of the organ. No correlation appears to exist between the numbers present in the right and left gonad. They lie approximately equidistant from the margins of the gonad and are quite widely separated from each other. The early gonia are relatively large spherical cells with a uniform mean diameter of 6.3 microns enclosing a sharply defined, open faced nucleus of a little less than 5 microns which is constituted of a minimal quantity of delicate chromatin strands and a prominent centrally located nucleolus. Their cytosomes are homogeneous and slightly acidophilic.

(2) Histogenesis of Somatic Elements in Post-larval Gonads (30 to 70 mm Fish)

The early provisional somatic constituents described above progressively differentiate as growth of the fish occurs, and with the development of fibrous elements the organ attains the histological appearance of early adolescent gonads.

The stromal tissues in gonads of fish between 30 and 38 mm in length have increased proportionally with the continued enlargement of the organ. It thus retains its solid appearance due to the density of the replicated cells, except in areas where it is permeated by the few small vascular channels previously described. The degree of vascularization does not noticeably increase, and the margins of the sinusoidal spaces remain void of endothelium. Various forms of early fibroblasts occur in the dorsal one-third to one-half of the organ where they are present to the exclusion of the gonia. Below this area the solid massing of the fibroblasts is irregularly interrupted by the presence of the germ cells. In the environs of the mesenteric attachment, the stromal fibroblasts exist as spheres, ovals, or similar robust forms with an occasional elongated or spindle type. They are not separated from each other by any visible intercellular matrix, and their cytology is similar to those found in 29 mm gonads. The ventral two-thirds of the organs contain a considerable number of gonia which have a modifying influence on the form of the associated fibroblasts. The connective elements occurring as ovals or spheres in this area are greatly diminished, and their presence is limited to the interstices between and at some distance from gonial capsule cells. The capsular cells evidence a greater degree of development than earlier. They have increased to some five or six layers and, although not sharply set off marginally from the outlying fibroblasts, the separation of the capsular elements from the general stroma is more pronounced than previously. The cells of the capsule lie in contact with each other, and their long axis is curved to conform to the gonial margin. They extend around each sex cell in numerous orbital planes so that they lie

at various angles to each other. A few indiscrete fibers oriented circumferentially have appeared at the outer and inner extents of the capsular investment thus tending to define somewhat the limits of the structure. Similar fibrous elements also exist in varying numbers throughout the organ, in general presenting no observable organization except that many are arranged in radial directions between the internum and the periphery. A principal cytological distinction between the capsular cells and the general stromal type of fibroblast is that the former possesses a cytosome which is noticeably more chromophobic than the latter type.

The epithelium investing the surface of the organ has not changed noticeably since the earlier stage. Because its cellular differentiation is static in this respect there still occurs the transitional pattern between its superficial cells and those subtending into the adjacent stroma as observed earlier.

A continuation of the organization and differentiation of the previously initiated events is manifest in gonads of post-larvae of 47 mm. Particularly noticeable is the substantial increase in the fibrous elements, and in some areas of the organs a possible precocious appearance of the initial stages of ovigerous lamellae, although the latter occurrence is normally delayed until later.

Because gonial cells now begin to appear in greater abundance in the more dorsal part of the gonad, which was originally occupied by large numbers of stromal fibroblasts, the population of these cells in this region has relatively materially diminished. Also, but to a lesser degree, the introduction of new generations of lightly staining gonia in the remainder of the organ, which is not equaled by an augmentation of the smaller, more intensely-staining stromal cells, causes the complex to appear considerably less dense than previously. The reduplication of the gonia has resulted in a considerable volume of the organ being occupied by these cells. In some parts crowding of such cells occurs so that they lie in close proximity with each other in the form of nests. The capsular cells associated with each gonium within a nest, although still concentrically arranged, are fewer than previously and usually are reduced to form one to three layers. The lesser number occurs particularly in the interstices between two or more closely associated gonia, while the surfaces of isolated gonia or the peripheral faces of a nest may be comprised of from three to four capsular cells. In this manner, gonia which are not closely associated with others possess a complete capsular structure of rather uniform thickness, but in other cases the thickness of the layer is inversely proportional to the degree of crowding. The orientation of the capsular cells is such that they encircle the enclosed gonium in diverse directions as heretofore. The capsule cells are not definitely stratified into distinct layers, and no organization occurs with respect to a stratification of the orbital directions of the individual cells.

Although the fibrils in gonads of 47 mm fish have increased numerically, they have not increased appreciably in thickness. Their diameters range from 0.4 microns to invisibility. Under these circumstances many cannot be readily identified and could not be clearly demonstrated with Cajal's connective tissue stain. The inability

to differentiate the fibers by this method suggests that they are embryonic or immature. Their frequency is greatest in the area of the gonial capsules and the vascular channels than elsewhere. In the capsular areas they tend to be oriented in a somewhat circumferential manner, conforming to the directions of the investing cells, although individual fibers are observed to pass from the internal to the external surface of the capsule and thence into the stromal material. There is no noticeable difference in the number or thickness of the fibers adjacent to the margin of the gonia in contrast to those at the outer extent of the capsule. At the margins of the gonad, in the zone underlying the superficial epithelium, they present no evidence of becoming organized into the *tunica albuginea*.

The weak connective tissue fibers associated with the small, irregularly shaped vascular channels permeating the stroma are roughly disposed in a vaguely circular investment with respect to what appears to be indistinct and incomplete endothelial walls. They are loosely arranged and since many of them continue outward into the general stroma, they do not constitute a recognizable theca. Although the existence of an endothelium is suggested, its cellular elements escaped confirming identification.

The primitive intragonadal cavity which will ultimately give rise to the discharge tubules for the sex products may or may not appear in gonads of 47 mm fish (Plate I, Fig. 4). When present, it consists of a medial vertical longitudinal cleft, which appears to have originated by delamination of the connective tissues. It extends from the gonoduct at the dorsal-cephalic end to about the posterior two-fifths of the organ. Thus in the female, the creation later of the original inter-lamellar spaces appears to represent caudal and transversely-directed extensions of this cavity. The mean transverse diameter of this cavity at its widest point is approximately 8 microns. The margins of the cleft consist of a layer of somewhat flattened, irregularly-shaped epithelial cells which may have many of the characteristics of the underlying stromal fibroblasts, between which no line of demarcation exists, including the interposition of basement membranes. The cells bounding these surfaces are noticeably irregular in their pattern of distribution, thus establishing an illusion that in local areas the surface is denuded. Although very small nuclei can be recognized, cytological details of these cells were for the most part not evident, and because of the obscurity of limiting cell membranes, their surfaces are not definitely discernible. The cytosome is greatly reduced, a state which is particularly pronounced in the apical zone lying above the oval or flattened nuclei.

The epithelium comprising the external surface of the gonad has not undergone further differentiation from the condition present in younger fish.

Except for the increase in numbers of stromal and capsular cells which occurs in gonads of 61 to 68 mm fish as an accompaniment of their growth, they are cytologically similar to those occurring in 47 mm fish. The most significant morphogenic development in these older gonads is the occurrence in some of the preparations of numerous cavitations. These cavities, the spaces of which are not connected or associated with the intragonadal sex ducts described above, and

Fig. 1

Fig. 2

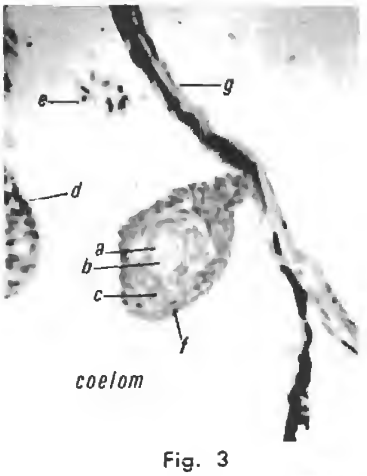


Fig. 3

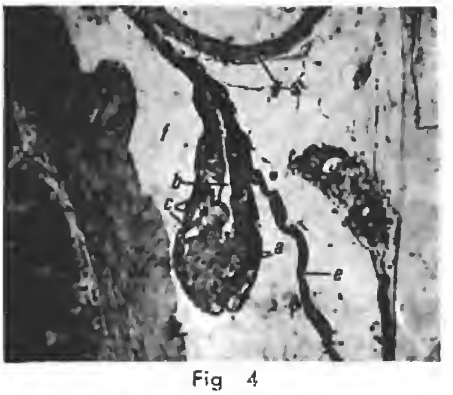


Fig 4

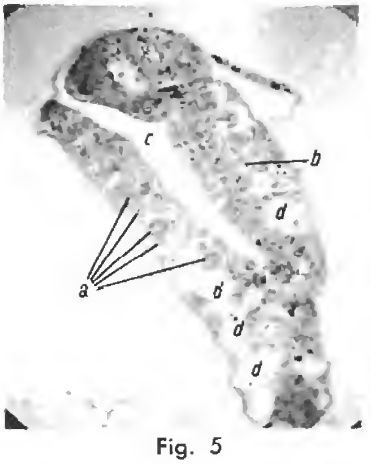


Fig. 5

PLATE 1

Fig. 6

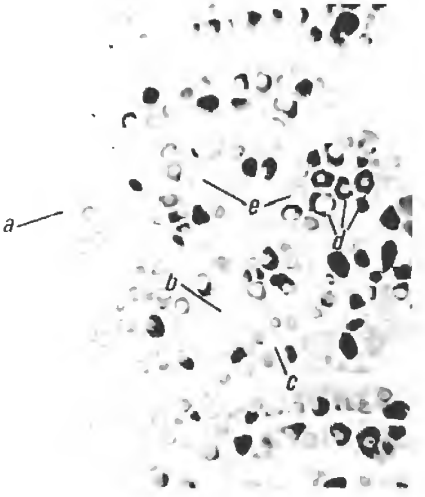


Fig. 7

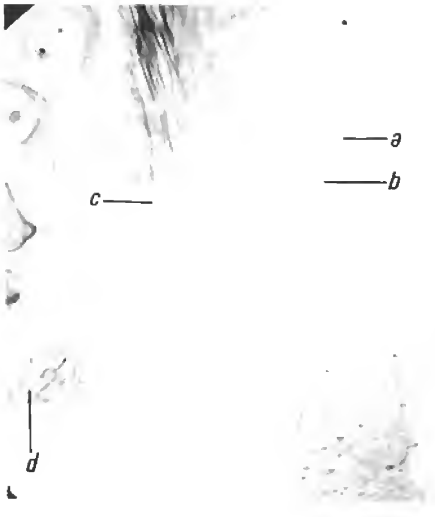


Fig. 8

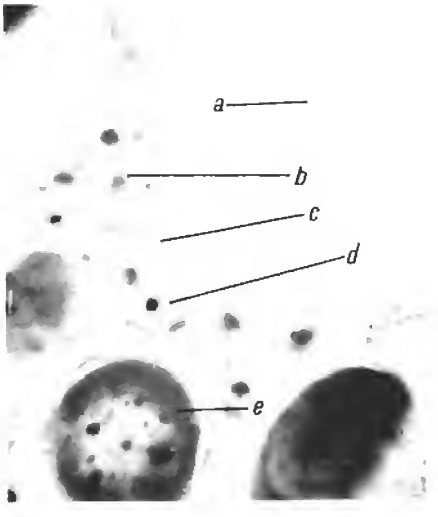


Fig. 9

PLATE 2

which are not vascular in origin, have a somewhat random pattern of distribution, although a slight preponderance is evident near the mid and ventral area of the organ. From a maximum of four to six found in sections from the mid anterior-posterior region of the gonad, their frequency diminished toward the terminal extents of the organs. The form of these irregular spaces varies from triangular to flattened compressed types, and includes numerous intermediate patterns. The compressed spaces have approximate mean widths of 6 microns and vertical lengths of 22 microns. Their anterior-posterior limits were not determined, although it was observed that a single cleft continues through a considerable number of sections. The cross-sectional area of the associated, less compressed, cavities varies from 8 to 16 microns for the larger forms to occasional smaller ones of about 5 microns in diameter. All of the cavities are bounded by a noticeably sharp acellular membrane constructed of a sheet of connective tissue fibers 0.4 to 0.6 microns thick, the sheet lying concentric with the margin. Here and there along its course, some of the fibrous components can be seen to extend into the stromal substance of the organ.

Noticeable deviations are manifest in the nature of the enclosed contents of these cavities. In some locations they appear to be devoid of formed material, while in other areas the lumen is occupied more or less by a finely granular oxyphilic substance which may or may not enclose a single spheroidal object. The size of these objects, when present, varies from three to five microns. In still other cavities, the spheroidal bodies are absent and the entire lumen is occupied by the granular substance. The origin of the granules could not be specifically ascertained, but there is reason to believe that they are the terminal stage of degeneration of the spheroidal elements. This is substantiated by the variability of the cytological structure of the spherodial bodies, and by the nature of the association of the granules with the larger bodies. It is found when large numbers of the spherodial bodies are studied that a small number possess a central inclusion of a pycnotic nature, which is thought to be degenerate nuclei. Regardless of whether or not the spheroidal mass contains an atretic basophilic nucleus, the predominant material evidences the characteristics of a granular oxyphilic cytosome. The cytosomal material is in no instance surrounded by a visible limiting membrane. Due to the absence of a distinct boundary, there occurs an imperceptible transition between the oxyphilic granular substance of the cytosome and the adjacent granules that extend outward to more or less occupy the lumen of the cavity. In certain preparations, it is found that there occurs among the oxyphilic granules lying adjacent to the cytosomal margin a small number of scattered basophilic granules. These are considered to be of nuclear origin.

The origin of the spheroidal objects could not be established with certainty. Those evidencing the least effects of atresia do not show a recognizable cytological relationship with the ichthyoid erythrocyte. In some respects they show a similarity with the gonia. On the other hand their smaller size and structure suggest that they may be derived from stromal fibroblasts which have become detached from the general stroma during the process of cavitation described below, and which then undergo lysis and degeneration. In the final stage after absorption of the oxyphilic granular residue, the cavity becomes oc-

cupied by tissue fluid.

The derivation and significance of the cavities is subject to various possible interpretations. Although they are suggestive of vascular channels, their size (in general, greater than the blood vessels), their shape (typically irregular), their position (particularly the medial laterally-compressed cavities), the complete absence of contained blood cells, and their inclusion of unusual cell types, constitute specific criteria distinguishing them from hemal channels. Since these spaces do not exist in earlier or later stage organs, they are not considered as artifacts due to the techniques used. Also their walls are of a nature that would preclude the existence of delamination. The evidence, on the other hand, postulates that their origin is precipitated by the atresia of individual gonia or entire nests of these cells. If such is the case, at least some of the associated capsular cells apparently become modified and migrate into the general stroma, where they cannot be distinguished from other fibroblasts while those most closely associated with the gonial surfaces come to occupy the cavity created by the degeneration of the gonia. In this process it appears that the connective tissue fibers originally associated with the gonial capsule remain intact and with some reorganization come to constitute the boundary of the cavities. A slight to moderate hypertrophy of the original intra-capsular lumen is also found to occur by the infusion of tissue fluids.

The fate of these gonadal cavities will digress later during the sexual transformation of the organ. Since they do not persist as such in the male they will not become involved in the establishment of the primitive semeniferous spaces while in the female they will contribute, by their coalescence with the medial cavity, to creating the ovarian lumens between the ovigerous lamellae.

Considerable marginal development occurs in the organs at this stage. The number and coarseness of the infra-epithelial investing fibers is materially greater than in earlier gonads. The constituent fibers are fundamentally arranged parallel to each other to form a moderately dense sheet of about 1.5 microns thick that sub-marginally circumscribe the organ. This constitutes the earliest definitely recognizable stage of the *tunica albuginea*. Throughout its course numerous individual fibers depart from this layer usually at rather abrupt angles to enter the general stroma, where they become unidentifiable from other fibers present. The provisional tunic is invested externally by a layer of simple epithelium which presents the appearance of incompletely covering the surface. The inadequacy of the layer is further indicated by the regressive condition of its cells, which are flatter than in some of the younger stages. The oval or spherical open-faced nuclei contain only minimal amounts of finely granular chromatin and for this reason appear as clear vesicles enclosed by the eosinophilic cytoplasmic background. Because apical cytoplasm is almost non-existent, the nuclei often protrude above the general surface of the layer.

Only one gonad was examined from a 70 mm fish. No appreciable histological advance was observed in the development of the potential gonoducts. Although no new cavities arising from the disintegration of gonia appear, many of the remnants of the older spaces

persist which are now devoid of particulate material. The medial vertical longitudinal cleft or space is somewhat wedge-shaped in a cross-sectioned profile, the greatest width occurring dorsally. In females the partial or complete separation of the ovarian stroma into separate lamellar folds occurs in gonads of older fish, but the positions where they will develop are fore-shadowed in 70 mm fish by shallow, somewhat triangular indentations along the right and left margins of the central cavity (Plate I, Fig. 5). Between adjacent indentations the medial faces of the solid gonadal tissues tend to round-up and project slightly into the central lumen in the configuration of a scalloped border. Thus, they resemble in a primitive manner the free or distal area of fully developed lamellae of adolescent fish. The delicate investment applied to the margins of the central cavity and its lamellar indentations is continuous and sharply defined. It consists of a few fine fibers intertwined with or associated with greatly flattened cells which cytologically are similar to some forms of fibroblasts in the stroma proper. Cytosomes of the cells are greatly reduced and their nuclei in sectioned material were frequently dense and rod-like. In the absence of a basement membrane the covering cells together with the fibrous components seem to merge with and continue into the general tissues of the stroma.

In addition to the interruption of the solidity of the gonad by the development of the above lumens, other events continue to contribute to diminishing the apparent density of the stroma characteristic of the younger stages. The change, which was initiated in 47 mm fish, is largely due to continued dispersion of the densely packed connective tissue cells that constitute the general undifferentiated stroma of the organ. The principal factor involved in bringing about the rearrangement and scattering the stromal fibroblasts is the continued increment of the gonial and capsule cells, which show a relatively greater chromophobia than the dense stromal elements. Since the gonia at this age occupy approximately half the volume of the gonad, the stromal fibroblasts are disposed either singly or more often in clumps of from three to five, each group thus being separated more or less from others by the nests of gonia and their capsule cells.

Although the total capsule cell content in these older gonads has increased materially, the relative rate of increase is proportionally less than the rate of increment of the germ cells. This state is reflected in a further diminution of the number of capsular elements associated with each gonium so that where as many as three to four layers of these cells were applied to the surface of gonia during the younger stages, the majority of the germ cells in 70 mm fish are invested with only one or occasionally two layers. Rarely is a germ cell encountered which has a three-layered capsular covering, but on the other hand, it is not unusual to observe gonia, which are crowded in nests, lacking capsular cells entirely at the interstices where two or more of them are intimately associated. The investing capsule cells and the barely discernible associated fibers cannot be said to constitute a follicle, although their conformation is comparable to the basic components that characterize the early stages of cells undergoing oogenesis in functional ovaries.

While a diminution is evident regarding the number of capsule cells which are associated with individual gonia, a reciprocal increase

occurs with respect to the number constituting the investment enclosing nests of gonia. Each such aggregate, which in sections may be observed to contain from two to five gonia, is imperfectly surrounded by three to more irregularly arranged layers of the typical capsular type cells. The layering condition is subject to many irregularities both regarding the relative position of the cells with respect to each other and the direction of their longest axis. In general, the direction of the long axis of these spindle-shaped cells conforms with the outer curvature of the gonial aggregate, although numerous exceptions occur. Thus, occasional cells may project outward at various angles into the unmodified stromal tissues, while other tend to penetrate toward the interior of the nest and become more or less involved in the capsular structure of individual gonia. Cytologically no distinction can be made between the capsular type cell constituting the outer boundary of gonial nests and those applied to the surface of a specific gonium.

Except for some increase in thickness, no marked development of the outer gonadal tunic has occurred in 70 mm fish. The superficial epithelial cells show a greater degree of discontinuity than earlier, so that the areas which appear to be acellular are more extensive. Existing cells are greatly flattened and have a minimum of cytoplasm. Their sharply defined nuclei have become dense and, as seen in sections, vary in shape from relatively long, straight rods to undulate forms. Cells of the outer layer are not noticeably separated from adjacent underlying fibroblasts by any structure resembling a basement membrane.

While regression is occurring in the epithelial layer, the over-all thickness of the gonadal covering is greater, however, than in younger forms as the result of a considerable increase in numbers of the fibers constituting the *tunica albuginea*. Because the interstices between individual fibers are generally greater than the thickness of the individual elements, the resultant arrangement suggests a loose type of irregular, coarse fibrous connective tissue. The peripheral face of this layer is fairly uniform and the internal surface is marked by the reflection of numerous fibers into the deeper gonadal tissues. Many of these elements leave the tunic individually or in poorly defined bundles of only a few fibers each which soon become lost in the stromal complex. More rarely, at somewhat irregular intervals, considerable numbers of the marginal fibers become organized into a relatively large, compact bundle or sheet, which projects a short distance into the deeper tissues. These bundles rapidly diminish in diameter and cannot be followed very far from their points of origin in the tunic. When encountered they usually appear as a tuft whose apex is inwardly directed along a radial axis. Their distribution in the gonad and their histological organization suggests that they represent an early stage in the formation of the core complex of the future ovigerous lamella or in the male the walls of the semeniferous channels.

Between the fibers of the *tunica albuginea* there occur two types of cells distributed at random and displaying various transitional states. Of one type, which represents the minority of the total, the thin-walled nuclei are characteristically elongated ovals or occasionally spindle shaped. In these chromatin material is not evident. The

other cellular moiety is marked by the presence of a heavy, spherical or broadly oval nuclear membrane enclosing an appreciable quantity of granular chromatin. The cyto-differentiation presented in the latter type is comparable to that occurring in certain fibroblast-like cells in the stroma. It is postulated, therefore, that these cells are of the nature of fibroblasts, while the other forms are presumptive myoblasts which, as stated subsequently, are known to occur in this area. No marked differences are observable in the size, density, form or tinctorial characteristics of the cytosomes of the two types of cells. The oxyphilic cytoplasm is finely granular or amorphous and evidences a marked similarity to the associated fibers. To a considerable extent the evaluation of their size and morphology is precluded by the obscuring effect of the fibrous elements.

(3) Size Increments in Primitive Gonads

After the germinative and supporting tissues become organized into a recognizable gonadal structure in larvae of less than 20 mm, the organ rapidly increases in size. The increments which occur present two interesting aspects, namely the overall relative rates of growth along the major axis of the organ in 23 to 70 mm fish, and also the sequential increments that appear during this period.

Because the minuteness of the organs found in fish of less than 60 mm constituted a problem in measuring intact and subsequently embedding and sectioning, their dimensions were determined from serial sections of the entire segment of the specimen bearing the gonads. In the larger fish, measurements of the organ were taken prior to sectioning, but after fixation. The sizes thus obtained in both types of cases, while less than they would have been in the fresh state, still provide precise data to compare relative growth trends.

Table II contains measurements of the longitudinal, vertical and transverse extents of fish whose fork lengths are between 23-70 mm. While the sample used was not large, a considerable degree of agreement exists in the data. From the data it is found that the overall rates of increment along each axis for the entire period for which measurements were made is 900% vertically, 606% longitudinally, and 330% transversally. In terms of changes in the size of the organ along its respective axes as a percentage of the fork length of the fish, the data shows the following: length 4.30 and 8.57; vertical 0.0043 and 1.3; transverse 0.17 and 0.17 where the first amount in each case is applicable to 23 mm fish and the second to 70 mm specimens. With respect to the magnitude of these gonad-fork length differentials and also the previously stated rates of increment of the respective axes show that the vertical growth of the organ not only exceeds the increment along its other axes, but that its development in this plane is relatively more accelerated than the body growth. The data also indicates that between the younger and older fish a constant relationship is attained in the transverse gonadal enlargement as related to fork lengths throughout the period, i. e. 0.17 per cent of body length in the case of both the 23 mm and the 70 mm specimens. The increase in its length relative to the body growth occurs at a rate intermediate between the rates applicable to the other axes.

Futher consideration of the growth of the organs during this period from the aspect of the phasic rates of development shows that

the length and transverse enlargements, when plotted as semi-logs, occur at a constant exponential rate relative to the increase in body length (Chart 1). Their vertical rate of increment, however, manifests two distinct segments. During the larval phase, i. e. in fish between 23-30 mm, the progression of vertical enhancement relative to the elongation of the body is considerably more accelerated than in fish in the 30-70 mm range. This diphasic rate of development is attributable to two factors. In part it is the result of the precipitative substention of the originally flattened germinal ridge ventrally into the coelom to establish the form of the organ. Also, in part, its early accelerated growth along this axis is due to dorsal-ventral elongation of the coelom as a result of similar changes in body form. This modification is observable in cross section profiles of larval bodies wherein it is seen in the youngest stages that its contours are more of a subcylindrical nature and that considerable relative flattening occurs when the specimens have grown no more than 10-15 mm in length. Suttkus (1956) has shown by plotting percentages of the depth of the body to the standard length for fish between 23 mm and 70 mm that a rapid increase in the dorsal-ventral axis apparently terminates at about thirty millimeters. As a result of this rather sudden extension in the depth of the coelom, room is provided for the gonad to push ventrally at a rapid pace as described above.

(4) The Gonia in Larval and Post-larval Gonads

The transformation of the primitive germ cells derived from the retroperitoneal areas into the gonial state appears to occur during the larval stage. The cytomorphosis of the gonia into early stage oocytes is, in general, held in abeyance throughout the post larval period, except for rare occurrences of a few young oocytes appearing precociously in the gonads of occasional 70 mm fish. In these instances, the maturation of the gonial cell into a characteristic first stage oocyte is incomplete and the cells involved appear to be intermediate between the original gonia and the young oocyte. Since the components of these gonads are not sufficiently developed and organized to sustain cells of the degree of maturity of an oocyte, it is believed that these occasional transitional cells either abort or that they are retained in this intermediate stage pending further maturation of the organ. The cytology of these metamorphosing cells is described in Part VI in conjunction with the gametogenesis of the cells in the functional ovary.

Following their initial appearance in the primitive gonad, replication of the gonial components progresses rapidly. Table III contains an approximation of the numbers present in a 100 micron segment at the mid-region of the organ, and the estimated numbers in the entire gonad for fish measuring between 29 and 70 mm. The method used in arriving at the number of cells/100 microns involved tallying all cells appearing in sections equaling a linear distance of 100 microns and adjusting this total by a factor reflecting the percentage of cells that would in probability be counted in more than one section. Estimation of the entire gonadal complement of the gonia, while not as accurate as would be obtained by an actual count of all the cells in the organ, is sufficiently reliable to demonstrate their rates of increment during these developmental stages. The form-

TABLE III

Number of gonia related to age of the post-larvae.

MONTH (1)	MEAN FORK LENGTH (MM) (2)	MEAN GONAD LENGTH (MM) (3)	MEAN NO. GONIA/100 MICRONS (4)	ESTIMATED TOT. NO. GONIA IN GONAD (5)
May	29 (2)*	1.74	5.6	487
April	32 (2)	1.24	9.7	626
May	34 (1)	1.22	8.0	488
June	47 (1)	1.92	20.9	2006
June	61 (1)	3.10	24.1	3735
August	64 (1)	3.50	106.7	18,672
August	66 (1)	3.40	182.9	62,086
August	70 (1)	6.00	219.1	65,730

*—Number of gonads included in sample.

ula L (in microns)/10 x No. cells per 100 microns x 0.5 provides a reliable approximation for the purpose for which the data is used. In the formula L represents the length of the organ; the denominator 10 adjusts the total micron length to the proportionate distance that cells were computed in the 100 micron segment; and the factor 0.5 is used to reflect the tapering of the organ from its mid region to its ends. While this factor may be slightly more or less than actually occurs, it nevertheless does provide consideration of the fact that the counts of gonia were made in the mid region of the organ where its vertical and transverse axes are greatest, and that these diminish to zero at the ends of the gonad. This computation also rests upon the assumption that gonial replication occurs at the same rate throughout the extent of the organ, which condition was verified by observation.

Taking into consideration the sample size, there appears to be a continuous augmention of gonial numbers during the interim from May to August. In 70 mm fish there occurs approximately thirty-nine times the number found per 100 microns in 29 mm fish, or if the estimated total number present in the gonad is employed to indicate the rate of replication of these cells, it is found that at the final stage for which data was accumulated the increase over the initial stage is about 135 fold. Since production of new generations of gonia is not confined to a 100 micron segment of the gonad but involves the entire organ, the percent of increase which can be derived from data in column 5 of Table III provides a much more representative picture of the events which occur during this period.

When the mean numbers of gonia per 100 microns, column 4, or

the total complement of the organ, column 5, of Table III, are plotted as semi-log functions of the fork length of the larvae and post-larvae (Chart 2), although some scatter exists, it is obvious that the rate of enhancement of gonial numbers equals or exceeds the rate of elongation of the fish. Initially, in fish ranging from 29 mm to approximately 54 mm, the production of new gonia appears to occur at approximately the same exponential rates as increase in body length. Thereafter gonial augmentation becomes moderately accelerated.

In summary, the data suggests that gonadogenesis during the larval period and the younger stages of the post-larvae especially involves the accelerated origin and organization of the non-germinal elements of the gonad that are necessary to sustain the germinal units. While these preparatory activities are in progress, the organ is incapable of producing or maintaining a large population of gonia. A visual study of the organs from the time they are formed by the entrance of the retroperitoneal elements substantiates the concept that their initial enlargement is principally the result of the creation of an abundance of stromal tissues coincidental with the establishment of blood channels and the intra-gonadal cavities. It is also logical to assume that gonial production is controlled by hormones derived from the capsule cells or from elsewhere, and that at this time they have not attained a sufficient level to implement rapid gonia production. Thus it appears that both morphological and physiological activities in the initial growth phases are primarily directed toward organizational activities and that subsequently in the older post-larvae they have reached a potential capable of inciting gonial production. Likewise the endocrine status in these older forms being further developed is more capable of responding to the tropic influences of temperature, salinity, etc., which in turn exerts an influence on the rate of gonia production. The assumption that the environmental factors regulate the rates of increment of gonia in these young fish is supported by a comparable situation regarding the induction of gametogenesis in mature fish, in which gametogenesis is somewhat held in abeyance during the spring and summer but accelerates during the fall and winter.

IV. SEX DIFFERENTIATION IN THE LARVAE AND POST-LARVAE

The transformation of the indifferent gonad into male and female entities occurs shortly after the larvae have entered an estuarine environment. In the Mississippi Sound, this event transpires normally in the months of April and May. It seems to require only a period of twenty to twenty-five days for its elementary completion after it is initiated. The entire interim from the inception of the organs to the establishment of the primitive sexual state is believed to occupy about four to six weeks as reflected by the growth rates of the fish. Relatively few exceptions to this schedule were observed in the 57 larvae and post larvae which constituted the sample for which data was accumulated. In one instance, a single June caught specimen appeared not to have initiated gonadal differentiation, and one larvae obtained in August was found to have gonads in essentially an indifferent form but evidencing a few characteristics of a young testis

TABLE IV

Sex Differentiation of the Gonad as Related to its Size and to the Fork Length of Larvae and Post-larvae[1/].

Fork Length (mm) (1)	No. in Sample: Indifferent (2)	Male (3)	Female (4)	Gonad Length (mm) (5)	No. in Sample: Indifferent (6)	Male (7)	Female (8)
20-29	14	0	0	.100-.99	3	0	0
30-39	5	1	0	1.0-1.9	11	1[3/]	0
40-49	2	0	0	2.0-2.9	0	9	0
50-59[2/]	—	—	—	3.0-3.9	0	17	6
60-69	0	18	4	4.0-4.9	0	5	5
70-79	0	4	0				
80-89	0	6	3				
Total	21	29	7	Total	14	32	11

1/—Gonad length ranges are not necessarily identical with fork length ranges appearing at the same level in the table.

2/—No specimens examined in this range.

3/—Gonad intermediate between indifferent and male. August caught specimen.

suggesting that it was in a transitory state. Since in both of these cases fork length and gonad measurements showed the fish to be comparable in age to the April and May specimens in which gonadal maturation had not yet become evident, it is concluded that the two fish in question did not reach the inshore waters until later in the season. Such a condition may be attributable to late hatching or having been retarded in their migration from the spawning area.

As shown in Table IV, the transition from the indifferent gonad to the immature sexual organ in a larval population is closely synchronized as regards the ages of its members, and also for the time interval required for its completion. Thus all individuals whose ages, as represented by fork length measurements, are (with one exception) less than 49 mm, exhibit no evidence of sexual metamorphosis. This coherency is also manifest, as shown in column 5 of the table wherein all the gonads of 1.9 mm or less (with one exception) have not advanced beyond the indifferent phase. This exception, regarding which it has already been pointed out, does not represent the normal pattern of development. Considering the slight degree of its transition toward

testicular tissue, one could justifiably consider it as being undifferentiated. The above data if used as a means of expressing the ages of the fish at this period, would indicate that, because of the immaturity and absence of sex differentiation, embryologically speaking, fish of 49 mm or less should be considered as being larvae.

Ovaries and testes are first recognizable in fish comprising the present sample in the 60-69 mm fork length range. Also as shown in the table, the ovaries have grown to between 2.0 and 2.9 mm in length. Embryologically this represents the post-larval phase of development that will continue for a relatively short period until gametogenesis is initiated. Because of the composition of the sample employed, there remains unanswered the question of the status of the organs in the 50-59 fork length group, which as shown by Table IV was not represented in the sample. However, from the pattern presented by the data, a postulation is permissible that sex first becomes manifest during the interval that the fish are in the 50-59 mm fork length range. This inference is derived from the conclusive break that separates the sexless gonadal state (40-49 mm range) and the inclusive appearance of males and females in the 60-69 mm class. Logically there should exist an interval intermediate between these two ranges in which the population should show transitional conditions of advancement, some members having undifferentiated organs while others exhibit male and female properties.

At the time that the larvae become post-larvae a number of gross and histological changes occur in the undifferentiated gonad that mark the onset of its metamorphosis into the primary ovary or testis. Immediately preceding this activity, the organ resembles that shown in Plate I, Fig. 3, but would evidence from three to eight gonial cells per section. Because of the variation which exists in the temporal occurrence of some of these activities, the events are more comprehensible if they are presented primarily in accordance with the specific changes involved rather than from the standpoint of the time sequence.

Although most of the significant changes that occur during this transitory phase are internal, some alteration in the contour of the organ is noticeable particularly if it is destined to become a testis. The more or less rotund undifferentiated gonad in this case undergoes a dorsal-ventral elongation so that in cross section it typically assumes a blade or arrow-head shape with the point or apex directed downward. Simultaneously its surface assumes a uniform entirety thus obliterating the irregularities and undulations that generally are present in the undifferentiated organ. In contrast, the ovary initially continues to remain somewhat globose and evidence an undulating surface. Although the ovary will later show a dorsal-ventral elongation, it is not as precocious as the testes in this respect. At the time that the internal changes indicate an ovarian transformation is in progress, its cross sectional profile more nearly resembles the conditions shown in Plate I, Fig. 4, rather than Fig. 5, which is from an older ovary. The gross changes in the ovary and testes which have been described are often not detectable in an individual organ possibly due to modifications introduced by fixation or embedding. They are most successfully demonstrated if a number of gonads are observed.

The internal histological modifications which occur at the time the ovaries and testes are evolving tend to fall into one or more of five criteria. Both the stromal substance and the sex cells become involved in these activities. Although the elements are undergoing an almost simultaneous transformation, they will be presented individually to simplify their consideration.

Very early in the transition from the indifferent gonad there occur noticeable changes in the relative amount and distribution of the connective tissues. In contrast to the testes in which these elements become relatively less prevalent and more widely dispersed, the incipient ovary possesses an abundance of very apparent connective tissue fibers and fibroblasts. This condition imparts to the ovary an appearance of coarse solidity whereas the structure of the testis is suggestive of a finer, more open nature. In this respect, the ovary seems to be less precocious than the testis in that it retains for a longer interval the characteristics of the undifferentiated organ. The compact arrangement of the ovarian stroma remains substantially unchanged throughout larval development and undergoes modification only after the definitive ovigerous lamellae make their appearance. Its density then decreases greatly as it becomes arranged in strands in the lamellar cores and as investments around developing follicles. This diminution of connective tissue fibers is accompanied by a material decrease in the fibroblast population. In contrast, following the initial relative decrease in the abundance of fibers and fibroblasts in the testis, there occurs no further diminution as development proceeds. Instead, a slight to moderate increase in their numbers accompanies the formation of the walls and the interstitial tissues of the semeniferous cavities in late post-larvae and afterwards.

Concurrent with the events described above involving an alteration of the quantity of connective tissues during the transition from the indifferent gonad, changes are also occurring in the arrangement and distribution of the components. Prior to the inception of sex in the organs, the stromal connective tissues appear typically as a solid mass. Fibroblasts are much more in evidence than the associated fibers. For the most part the cells and fibers are randomly arranged in the organ except in areas closely adjacent to the gonia where they form a weak circular investment. In the earliest phase of ovarian metamorphosis, some of the fibers seem to coalesce and undergo a vague rearrangement to form what appears, in a cross section, to be radial strands. When first initiated, only one or two such radial strands occur in a section. Their number soon increases to as many as six to ten per section. Considerable variability exists regarding the number of fibers, and therefore, the thickness, that constitute the several strands or for that matter the thickness of a single strand along its course. Although they are in general oriented radially, many deviations in direction occur along their course. Frequently they appear to be discontinuous. These strands are originally very poorly delineated from the general mass of unorganized stromal tissues which separates them. Other fine fibers present in the stroma pass into and out of the strands. The significance of these events is that they foreshadow the subsequent origin of the ovarian lamellae and will constitute the source of the connective tissues for those structures. In these later activities a radial form of delamination will seg-

regate the general pool of stromal elements, which still exists when the ovary comes into existence, into the separate lamellar components. In the case of the evolving testis, these radial strands never make their appearance.

Not only do the connective tissue components undergo a degree of radial orientation as described above, but concurrently with the onset of transition of the indifferent organ they show a distinctly different pattern in their relationship to the oogonia and the spermatogonia. Prior to gonadal metamorphosis, the gonia were encapsulated in the manner shown in Plate I, Fig. 3. In the case of ovarian evolvement, an increase occurs in the number and density of encircling connective tissue fibers without a comparable augmentation of fibroblasts. The primitive follicle wall thus formed becomes relatively thick. Also, because of some increase in the size of the enclosed oogonium, enlargement of the follicular investment occurs so that its diameter approximates 11 microns. The picture thus presented of the follicles in the differentiating ovary is one of prominence of size and follicle structure. The connective tissues of the early differentiating testis which are associated with the spermatogonia exhibit an entirely different arrangement than occurs in the ovary. In the male organ delicate fibers ensheath the numerous gonia, which because of their much greater numbers lie in closer proximity to each other than do the oogonia. Often only one or two layers occur between adjacent spermatogonia. Instead of each germ cell having its own complement of investing fibers as occurs in the differentiating ovary, the filamentous elements in the testis ramify throughout the organ and become associated with numerous spermatogonia. Thus with respect to a specific germ cell, the fibers present may encircle it for a greater or less distance and then singularly or in small strands of two to three extend outward between the interstices of other near-by gonia where they may become incorporated temporarily into their investments. Under these conditions it is impossible to locate the beginning and ends of the fibers. The general impression presented by these ensheathments is that of a compact hexagonal lattice work in which the inner faces are circular where the fibers lie on the surface of the gonial cells. Shortly after the origin of the investments of the individual gonia, the testis produces a number of slightly heavier fibrous encasements each of which encloses an aggregation of from six to twelve spermatogonia. Only three or four of these encasements may appear in a cross section of a testis in its earliest phases of differentiation, but the number increases rapidly thereafter. Each is constituted of fibers which are cytologically similar to and of the same size or very slightly heavier than those applied to the surfaces of the individual spermatogonia. The encasements lie directly on the surfaces and in contact with the investments that surround the more peripheral spermatogonia. In this position, they receive fibers more and give off these elements to the nearby gonial investments. The events occurring in the testis at this period represent a foreshadowing of the subsequent transformation of these pockets or nests of gonia into the semeniferous spaces containing the sex cells and the contribution of the surrounding encasements to the interstitial tissues and the walls of the semeniferous cavities.

An important consistent feature appearing very early in the dif-

ferentiation of the gonads is the number, arrangement, and cytological properties of the oogonia and spermatogonia. Their frequency in both sexes shows some increase with the onset of sex transformation. In sections of the indifferent gonad, from three to eight of the sex cells are visible. Computations derived from data obtained from counts of the cells present in sections of ovaries undergoing transition indicate that their frequency is between one hundred and one hundred twenty-five per square millimeter. In a comparable age testis their numbers are approximately 3400 per square millimeter. As a result of the phenomenal increase in the male, the cells lie closely adjacent to each other to the exclusion of most of the stromal elements, a condition which is quite distinctive to the wider spacing of the cells in the ovary and the inclusion of considerable amounts of connective tissues in the intervening spaces. Cytologically, the chromophobia of the spermatogonia in the presumptive testis is more pronounced than occurs in the oogonia. This factor associated with the greater number of cells present in the testis imparts to it a kind of translucency that is not observed in the female organ. Other than the modification of the staining properties, no other distinguishing cytological features can be detected in the gonial cells of the two sexes.

Transitional ovaries furthermore consistently exhibit a pattern of stromal cavitation that is not found in the testes. Their numbers and arrangement show considerable variation. Initially only from one to four are visible in a tissue section but with the continued growth of the organs new ones arise at irregular intervals. As many as twenty-one such spaces have been counted in a section of a 3.2 mm ovary. When first detectable, they are about the same size or somewhat smaller than the oogonia. Their slightly irregular margins are bounded only by fibroblasts and rarely by a stromal fiber that incompletely encompasses the surface. Shortly after the cavities have made their appearance, they increase in size at rates exceeding that of the growth of the organ as a whole so that they impart to the tissues a sieve-like aspect. This conditilon is illustrated in Plate I, Figs. 4 and 5, in which views only a moderate degree of cavitation is evident. As the spaces enlarge, they assume various shapes and their margins become more irregular. The peripheries of the more advanced ones become invested with a detectable but not pronounced fibrous connective wall. The contribution which these spaces make to later ovarian morphogenesis is considered below.

While the stromal cavities are arising in the manner described, a second form of ovarian cavitation originates along the medial longitudinal axis. This activity is initiated near the dorsal cephalic pole of the organ, a little below the insertion of the mesentery. It rapidly extends ventrally and caudally in the mid-plane resulting in the separation of the ovary into right and left components except in an area of its ventral margin (Plate I, Figs. 4 and 5). This separation occurs along a poorly organized vertical longitudinal sheet of connective tissue, the elements of which remain adherent to the newly created faces of the stroma. Almost as soon as it comes into existence, its margins begin to evidence a few indentations in the direction of the lateral stromal material. Both the number of these recesses and the amplitude of stromal invasion increase as morphogenesis proceeds. Whereas the size of the apertures and the channels of the original

indentations are noticeably broad, they progressively narrow as the gonad becomes more completely differentiated. Ultimately they come into contact with and open into the numerous cavities described above that occur in the compact stroma. By this means the original channels are extended to the ovarian tunic. (Plate II, Fig. 6). In actuality what appears to be channels in sectioned material, exist in the form of clefts. The faces of these transverse clefts are not particularly parallel to each other, so that the enclosed spaces are of variable widths. New generations of clefts arise from the previously established spaces and thence extend toward the margins of the organ in a root-like manner. The various clefts intercept each other thus partitioning the stroma into irregularly shaped sheets and finger-like columns. The bases of the stromal columns thus produced lie on the *tunica albuginea* and their free ends extend into the central lumen of the organ. Each of these stromal columns contains several oogonia in addition to the connective tissue elements. The resultant structure constitutes a primitive ovigerous lamella, which from its manner of origin is not of uniform thickness throughout. The original lamellae are somewhat massive. However, due to some continued production of secondary and tertiary clefts from the primary channels, the early massive type of lamella becomes converted later into more elongate forms. Two additional factors which contribute to their elongation is their extension by terminal growth into the central cavity of the ovary, and by growth increments of their entire lengths made possible by lateral expansion of the gonad.

Except where otherwise stated, the events described in the preceding paragraphs have their origin in the earliest phases of the differentiating gonad. After they have resulted in the establishment of the basic architecture of the ovary, the oogonia, which to this time have played a passive role, begin to evidence activity in the direction of gametogenesis. This normally occurs during the early summer while the fish are still late larvae or post-larvae. Plate I, Fig. 5 of a very young juvenile shows the advancement of the oogonia into Stage I and Stage II oocytes. In this illustration, the Stage I cells are barely visible while the Stage II oocytes, of which there are only two or three present, are the larger cells with a fairly distinct profile. The number of these cells is greater in the figure than in the younger fish.

With the completion of the activities described, a definitive functional ovary is brought into existence. Its further development involves a continuation and refinement of the connective tissues and the arrangement of these components into a functional pattern, the continued growth and organization of the ovigerous lamellae concurrent with movements or migrations of the sex cells to superficial positions along their surfaces, and the augmentation to the earlier population of oogonia.

V. ORGANOLOGY AND HISTOLOGY OF THE NON-GERMINAL ELEMENTS OF THE DEFINITIVE OVARY

Continuing morphogenesis and progressive enlargement of the organs which occur subsequent to the initial organization described above culminate in the establishment of the functional adult ovary.

Fundamentally these changes in the non-germinal components consist of refinements associated with growth of the ovarian *tunica albuginea* (Plate II, Fig. 8) and further supplementation and growth of the lamellar folds.

The outer tunic becomes quadripartite comprised of a superficial epithelial investment, a prominent connective tissue sheath, an internal muscular layer, and an internal facing. The entire complex increases in thickness from about .04 mm in gonads ranging between 15 to 20 mm in length to about .08 mm in 25 to 35 mm gonads. At maturity in gravid ovaries this thickness diminishes slightly, due to stretching in response to internal pressures developed by the burden of mature ova.

The outermost component of the theca consists of a layer of chromophobic simple squamous mesothelial cells whose mean thickness is 4 microns or less. The presence of this layer in illustrated in Plate II, Fig. 8, as a thin undulating homogenous line lying on the surface. The small, widely spaced pycnotic karysomes characteristic of the epithelium of 15 to 20 mm organs become progressively smaller and less frequently encountered by the time the organs have attained a length of 25 to 30 mm. Still later, as in gravid ovaries, the cellular elements appear to be completely absent, and in their place there is present material that suggests a fibrous origin. In small ovaries a moderately distinct basement membrane of about 0.2 microns thick underlies the superficial cells, but with further growth of the organs it progressively diminishes until it becomes no longer distinguishable.

The sub-epithelial connective tissue layer constitutes the principal component of the tunic. As in the case of the superficial epithelium, its maximum development occurs during summer and fall while oogenesis is in its earlier stages, and diminishes thereafter as the organ attains the gravid state. It is comprised entirely of relatively coarse fibers the majority of which encircle the ovary in a plane perpendicular to its long axis as shown in Plate II, Fig. 8. This illustration was made from a longitudinal section and therefore presents the cut ends of the elements. The layer is not of uniform thickness in all areas. In some places it may represent as much as three-fourths or four-fifths of the overall thickness of the tunic while at other places it may be reduced to about a fourth of the thickness. Often it reaches its highest state of development at points where inter-lamellar septa arise from the tunic as seen in the figure. Although the general pattern of the fibers is one of gonad encirclement, it can be seen in sections passing through the tunic in the manner of a chord through a circle that many of the fibers extend around the organ in oblique directions. The irregularity of fiber orientation is also pronounced in its inner face at points where the inter-lamellar septa arise. Here many fibers deviate from the normal circumferential pattern to enter the septa. Their entrance into the latter structure may or may not be organized in the manner of parallel strands. A heterogeneous arrangement is illustrated in Plate II, Fig. 8, where the fibers appear as colorless elements intermingled with the darker muscle cells. Many fibroblasts are associated with the fibrosal investment and are depicted as small black dots in the photograph.

In addition to its participation in the creation of the inter-lamellar

septa, it gives rise to rather delicate collections of fibers that pass into the cores of the lamellae so as to constitute the principal component of the intra-lamellar connective tissue. Two complete strands are visible in Plate II, Fig. 7. These intra-lamellar entities become less indentifiable as the ovary matures.

Lying under the fibrous investment there occurs the third layer of the tunic. It is comprised principally of smooth muscle with a minor contribution of fibrous elements. The identity of the contractile elements was established only in sections stained with Cajal's or Flemming's. This tissue is present in all ages but appears to reach its zenith in juvenile, adolescent, and young mature ovaries when its contribution is relatively greater to the other tissues than in gravid and spent organs. Although the muscle cells do not present a unified alignment with each other, the majority are oriented so that their length coincides with the anterior-posterior axis of the organ. This pattern, as shown in the longitudinal section (Plate II, Fig. 7) is interrupted where the inter-lamellar septa arise. At the point of origin considerable disorganization usually occurs which is regained somewhat in the deeper sections of the membrane. The rigid, spindle shaped cells are oriented in the usual overlapping pattern characteristic of organized smooth muscle. As far as could be determined, they are similar in size whether they reside in the tunic, the interlamellar septa or in the core of the lamella. They have a long axis of about 35 to 40 microns and a mid-point transverse diameter of about 4 microns. The acidophilic cytosome is homogeneous and it not surrounded by a distinct membrane. Elongated rod shaped nuclei measuring about 8 microns in length lie in the cell center. Enclosed in a distinct nuclear membrane is a moderate quantity of finely granular chromatin suspended in a colorless nuclear sap.

The principal vascular elements of the ovarian circulation lie in the outer areas of the *tunica albuginea* and consist of right and left longitudinal channels situated parallel to and slightly below the line of insertion of the mesovarium in which approximate position they were present in the differentiating ovary. From these many arterial and venous tributaries arise and pass in a general ventral direction in the superficial part of the tunic meanwhile giving rise to prominent segments which invade the inter-lamellar septa, and also numerous units that ramify throughout the tunic or enter the bases of the lamellae where they ultimately contribute to the capillary system that has an extensive distribution in the cortical and sub-cortical areas of its investment. The largest segments normally consist of closely asociated venous and arterial channels.

Unlike the other elements of the *tunica albuginea* that show variable degrees of regression with the advancement of the sex cycle, the hemal system with two exceptions is maintained in a state of moderate to high development throughout the year. Its prominence increases in all areas of the ovary from a little after the beginning of oocyte maturation to about the onset of yolk formation. At that period many of the major channels lying on the ovarian surface appear to become more displaced toward the exterior as the tunical tissues become thinner. The condition with respect to these vessels is maintained throughout the remainder of the period that the eggs remain in the ovary. Likewise the vessels that enter the inter-lamellar septa

do not show signs of regression. On the other hand, the smaller vessels that course through the tissues of the tunic and those that enter the cores of the lamellae are either lost from view or diminished in numbers shortly after the beginning of yolk formation until the completion of ovulation after which they begin to reappear as absorption of the empty follicles progresses. This follicular invasion by the terminal segments of the system is illustrated in Plate V, Fig. 20.

The arterial channels that course through the tunic and the interlamellar septa are invested with a dense outer layer of what appears to be smooth muscle cells which are associated with an inner layer of coarse wavy connective tissue suggestive of elastic fibers. This fibromuscular complex is oriented circumferentially in from two to six indistinct layers according to the caliber of the channel. The pattern becomes less regular as tributaries leave the larger channels and ultimately disappears entirely. Histologically the arrangement does not establish a true vascular wall as normally recognized in higher forms since it is not distinctly separated from the tissue elements of the adjacent tissues, and because cytologically its components are similar to others comprising the tunic or inter-lamellar septa although the vascular contractile elements appear to be shorter than those present in adjacent non-vascular areas.

The fourth and innermost layer of the *tunica albuginea* is not of constant occurrence. In young adolescent ovaries it consists of a very delicate layer, the entire thickness of which is not more than 0.2 microns, comprised of a few greatly flattened, fibroblast-like cells associated with fragile fibers. When present it is continuous along the inner face of the tunic except at points where intra-septal and intralamellar connective tissue arise from the tunic. In these areas, it becomes applied to the surface of the reflections, but soon becomes indiscernible. At this stage its cells are smaller than the contiguous contractile entities that lie above them in the tunic. They do not possess definite margins and evidence no cytoplasmic architecture. Their dense small nuclei are flattened in the plane parallel to the surface of the membrane. Later, in ovaries which are approaching maturity, this membrane undergoes radical alterations at the interstices between the basal origins of the adjacent lamellae. This transformation brings about a progressive reduction of the fibrous material accompanied by varying degrees of fragmentation and a superficial inclusion of some of the parts in the adjacent muscular layer. Recognizable cells are now characteristically absent in these regions, the areas being occupied by a peculiar form of vacuolated tissue. The ovate-quadrate vacuoles, which become more numerous as ovarian maturity is attained, enclose a minimal amout of unidentifiable material which stains faintly with eosin. They are approximately 3 to 5 microns in size. The vacuolated structures lie adjacent to each other and increase in depth from one row during the initial period to about three imperfect rows at the completion of the process. The material incorporated in their margins is moderately acidophilic and presents the appearance of being shredded. The mean thickness of the vacuole walls is about 0.5 microns. Where these vacuolar entities lie adjacent to the muscular layer, fiber fragments of varying length and thickness are randomly disposed, forming a loose bond between the two. Their aspect is not of a regressive nature. Because of the

correlation of their development with maturation of the oocytes, they apparently play a role in the process.

Arising from the inner face of the *tunica albuginea* are the ovigerous lamellae characteristic of teleosts having a cavitated ovary. An account of their origin and early development in young differentiating ovaries has been given above. Their subsequent numbers and morphology are related to the age and sexual phase of the ovary. In adolescent organs the few lamellae present arise from the *tunica albuginea* by a relatively broad base and thence extend inward toward the ovarian lumen in the form of a hyperbola whose margins are relatively free of undulations or indentations. Their maximum inward excursion varies from about one and a half to two and a half times the distance across their bases. With growth of the ovary, lamellar elongation is evident, a condition which, together with an increase in their number, greatly modifies their form. They characteristically extend as much as two-thirds to three-fourths the transverse diameter of the organ so that their terminal portions interdigitate with each other. At the basal attachment of older lamellae to the *tunica albuginea,* the transverse extents become relatively narrower in proportion to their length so that they appear to arise more abruptly and have a greater uniformity in diameter throughout their length. Their profiles in sectioned material are marked by undulations and indentations on their lateral margins and by the impingement of neighboring lamellae (Plate II, Fig.7). Due to extreme distention of the lamellae in gravid ovaries, inter-lamellar spaces are reduced or obliterated so that their margins become applied to each other in a great variety of patterns dependent upon the conformation of adjacent lamellae. This condition also occurs during an interval following ovulation prior to absorption of the follicular remnants.

Lamellar surfaces are basically invested with varying amounts of loosely associated fibrous connective tissue, fibroblasts, and occasional contractile elements which are without specific organization. The abundance of these components is inversely proportional to the size of the lamellae. During late spring and early summer ovarian activity is at a seasonal minimum and it is at this period that the lamellar investment attains its greatest degree of development (3 to 5 microns thick). As the organ approaches the climax of its seasonal cycle the lining becomes less apparent, and in scattered areas only occasional strands of loose connective tissue are present. The reduction and disappearance of the lamellar investment is probably an important part of the morphogenic changes designed to weaken the superficial tissues constituting these folds to facilitate the escape of the eggs at the time of spawning. The principal factor involved in the reduction of the covering membrane during this period appears to be of mechanical origin resulting from the pressure hypertrophy of the ovigerous lamellae brought about by the burden of the enclosed ova.

Small vascular channels ramify throughout the marginal lamellar surfaces of maturing ovaries. Present are capillaries, terminal divisions of small arteries, and collecting veinules. In non-gravid organs the smallest of these channels occupy a more superficial position than the larger channels and are covered only by the thin investing lamellar membrane. Capillary abundance is such that they are seldom

separated from each other by more than 25 to 30 microns. The larger channels occur at various levels a little below the lamellar surface and in the proximity of developing oocytes. They are circumscribed by poorly organized triangular or irregular condensations of connective tissue fibers, some of which continue into the deeper stromal material of the lamellae either individually or as coarse strands. In gravid and newly-spawned ovaries the fibrous investment becomes reduced and evidences less organization. At the same time, a number of new channels appear within the lamellae and many of the superficial ones disappear. It could not be determined with certainty whether the new ones are of recent origin or whether they represent the previous channels that have moved deeper into the stromal substance. The lamellar blood supply is derived from major arteries and veins located in the *tunica albuginea,* from which ramifications continue through the core of the lamella and thence to their more peripheral areas.

The general stroma (Plate II, Fig. 7, and Plate VI, Fig. 22) of the lamella is comprised of a minimal quantity of loose, irregular connective tissue (except where it is associated with follicles), a central sheet or band of smooth muscle which also contains elements of fibrous tissue and occasional fibroblasts. The tissue elements comprising the lamellar core are continuous with the muscular and fibrous layers of the *tunica albuginea.* This intra-lamellar reflection attains its greatest development during the initial stages of oogenesis at which period it transverses from one-half to three-fourths of the length of the lamella. As it extends distally into the lamella it displays considerable variability in thickness and often presents an undulating appearance. These undulations, in general, conform to the folding or bending of the entire lamella, and are thus more evident in ovaries in which a crop of eggs are maturing than in juvenile or quiescent organs. Also, during the period that oocytes are ripening, the muscular core can be seen to develop on its lateral surfaces tangential tongue-like projections comprised of one or more muscle cells in which the long axes are oriented in the general direction of the periphery of the lamella. The number of these muscular projections in an individual lamella varies from none to four per oil immersion field, the increased frequency usually being associated with enlarged but not gravid lamellae. The extent to which these laterally directed processes project peripherally, although variable, is considerably short of the lamellar margin and in most instances they terminate between a fifth and a half of this distance. Great diversity exists as to the profile of these bundles so that they are encountered in sections as compact, simple, flame-like projections tapering distally, or irregular, branched structures whose thickness may vary along their course. At their extremity the individual muscular elements lose their intimate association with each other, in which case their distal ends project individually or in small groups of two to six cells each into the adjacent, loose connective tissue. The terminal arrangement thus presents a considerably frayed conformation whose individual entities may be rigid and straight or arched, the free ends of which become more or less intimately associated with the nearby fibers of the connective tissue. In gravid lamellae, morphological distortions of their central muscular cores have culminated in altering the arrangement of the components

to a state in which the cells are disassociated to a greater extent.

The connective tissues between the central core tissues and the lamella margins occupy the entire internum which is not devoted to the oocytes, vascular channels and the delicate muscular strands. The fragile fibrous elements usually appear singly, coursing indiscriminately and irregularly for short distances in the manner of a primitive form of areolar connective tissue. A few are visible in Plate II, Fig. 8, in the clear area near the inter-lamellar septum. Because of their paucity, the areas surrounding the oocytes and the internal musculature contain considerable quantities of tissue fluids. The abundance of this fluid is of material significance in providing an exchange mechanism between the cells in the lamella and the capillaries which are for the most part in the superficial areas. The frenquency of the fibers and the associated fibroblasts is variable according to the age and sexual phase of the organ. Their density is relatively greater in young or inactive organs than in gravid or spawned ovaries. Although the numbers and arrangement of the ramifying fibers are such that a direct and continuous relationship cannot be established between them and the contractile elements residing in the lamellar core or those extending peripherally from the core, their presence and general disposition is such that they appear to be capable of diffusively conveying contractile tensions originating in the muscular tissues to the various areas of the entire structure and its contents. The associated fibroblasts of the areolated connective tissue are small, usually irregular in shape and are promiscuously disposed. A minimal quantity of homogeneous acidophilic cytoplasm invests their elongated, oval and compact nuclei.

The dynamics of the muscular and fibrous elements of the ovary appear to be directed toward the effective performance of ovulation. The culmination of this activity is accomplished by the rupture of the follicle, at least in part, attendant to the application of external pressures. These forces originate through the simultaneous interaction of the muscles of the *tunica albuginea* synchronized with contractions of the cells embodied within each lamella, augmented by a shortening of the transverse axis of the ovary resulting from contractions of the cells in the inter-lamellar septa. This culminates in compression of the intra-ovarian tissue fluid and more significantly the fluids of the areolar spaces within the lamellae, thus assisting the rupture of the follicles. Contributing to release of the ova it also appears that the activity of the intra-lamellar muscles at this time is transmitted to the connective tissue investment of individual follicles so as to decrease its effectiveness in retaining the mature eggs. This system, in which a generalized pressure is exerted throughout the lamella and thence applied to the follicle and the enclosed egg, plus the selective action of loosening the follicular investment by the contraction of intra-lamellar muscle cells, permits considerable selectivity in the intermittent spawning of ova from the different lamellae or areas within a single lamella over a substantial period of time, as is thought to occur in *B. patronus* in the Gulf of Mexico.

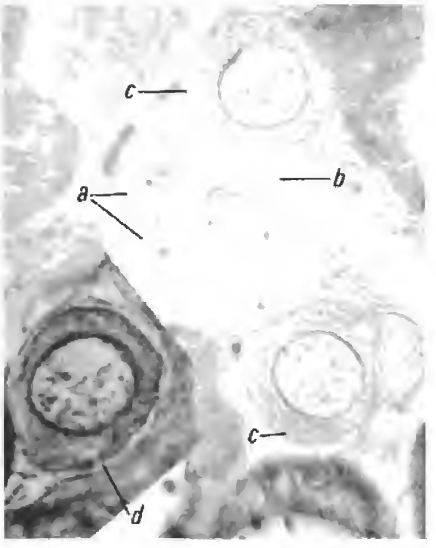


Fig. 10

Fig. 11

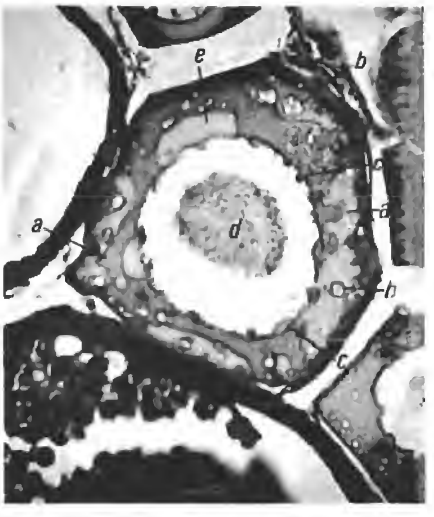


Fig. 12

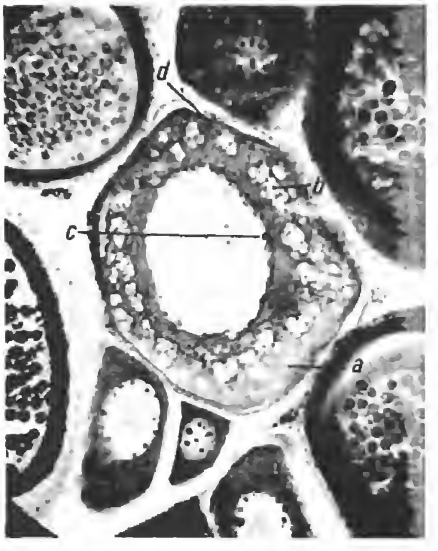


Fig. 13

PLATE 3

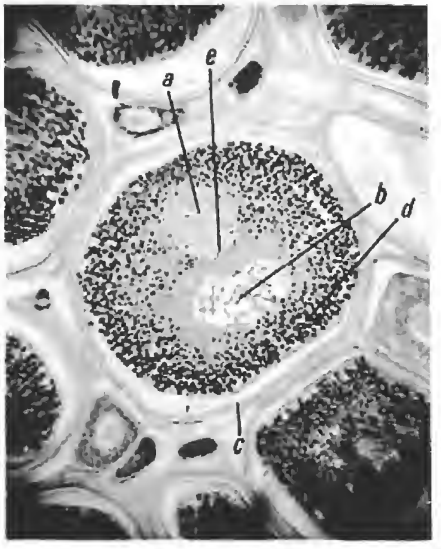


Fig. 14

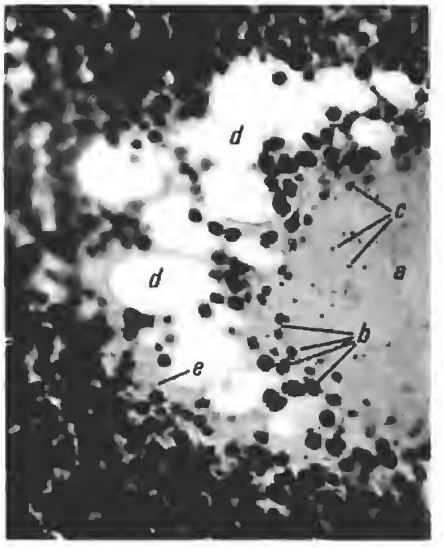


Fig. 15

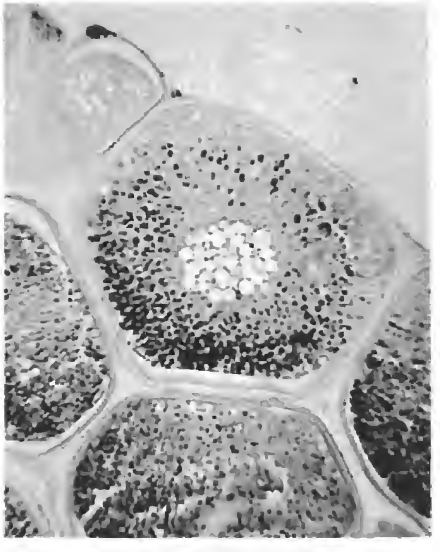


Fig. 16

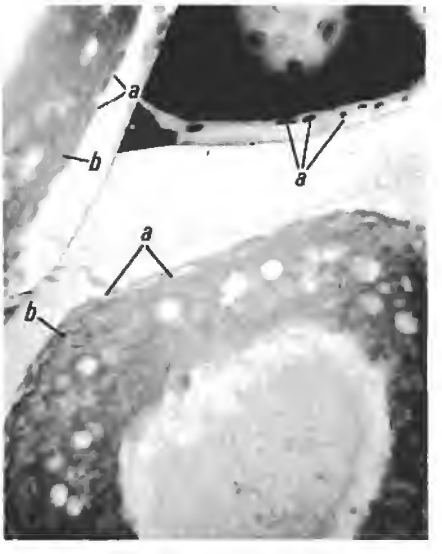


Fig. 17

PLATE 4

VI. HISTOLOGY AND CYTOLOGY OF THE GERMINAL ELEMENTS OF THE DEFINITIVE OVARY

Many methods have been used by various investigators in classifying the developmental stages of fish oocytes. In the studies conducted by the author, six maturation intervals are recognized. These are based on nuclear and cytosomal characteristics which are specific for each interval. The principal distinguishing features of each stage are:

Stage I. Undifferentiated oocytes not far removed from the oogonia.

Stage II. Oocytes evidencing meiotic differentiation.

Stage III. Oocytes undergoing initial lipogenesis.

Stage IV. Oocytes in which provisional yolk is being produced.

Stage V. Oocytes evidencing nuclear absorption, and concurrent production and maturation of vitelline elements.

Stage VI. Mature oocytes with a central oil globule.

Stage I: The cells constituting this stage are interphase or proliferating first phase oocytes. A number of constant characteristics specifically identify this period of development, principally the frequent disposition of the cells to be organized in groups or nests, the presence of a single prominent nucleolus, vague or indistinguishable cell outlines, a delicate serrate karyotheca, and their size. Cells of this age are shown in Plate II, Figs. 7 and 9. Figure 4 which is from a June specimen with an ovary length of 1.92 mm shows large numbers of these cells. They are the smallest cells visible. Figure 9 shows seven of the oocytes in more detail.

These oocytes are always oriented near the margin of the ovigerous lamella, where they are more or less encapsulated by a delicate, fibrous form of connective tissue. In adolescent ovaries and those of mature fish which are in a state of reproductive dormancy, they are typically organized into aggregations at locations where the marginal investing lamellar connective tissue is reflected into the interior of the ovigerous lamella. From three to ten such cells present themselves in each nest-like location in sectioned material. As the ovary begins to respond to oogenesis, the number of these early stage cells within a nest progressively diminishes as they migrate into the internal lamellar matrix or under the margin of the lamella. Early oocytes may also occur singly at irregular intervals in the lamellar investment which, due to its thiness at such points may be deflected somewhat as a slight elevation above their outer surfaces.

Stage I cells are present in greatest numbers in non-functional ovaries decreasing progressively throughout oogenesis until they cannot be found after the completion of yolk formation. In functional gonads of the large scale menhaden of the Gulf of Mexico, they do not appear during the period from October to February. A similar rhythmic behavior of the early sex cells has been identified by a number of investigators, and raises the question of the source of the next generation of oocytes. This matter has escaped elucidation although

many concepts have been advanced. It does not appear to be involved in the viviparous teleost, *Neotoca bilineata*, in which Mendoza (1939) found that nests of these cells occurred abundantly at all times, although a considerable decrease in numbers developed during the latter part of gestation. Wheeler (1924) admits that he is uncertain of the annual origin of a new crop of eggs in the dab, *Pleuronectes limanda*. He was unable to find any trace of mitotic division of oogonia, of oocytes or stages in the formation of oocytes, although, as soon as mature eggs begin to shed, small oocytes begin to appear. The yearly periodicity in the appearance of these cells was considered by Wallace (1903) to be from the ovarian epithelium and that the whole nest is derived from a single mother cell. His postulation is based upon his interpretation of the studies by Forchammer, Rathke, and Stuhlmann in the absence of his own direct observations. Some ambiguity also is introduced by his failure to identify specifically the nature or location of the terminology "ovarian epithelium." Hickling and Rutenberg (1936), in considering the ovary as an indicator of the spawning period, elude the question of the periodic origin of a new generation of oocytes by assuming there exists a general egg stock from which a quota is withdrawn each season. Their cytological description of the "egg stock" establishes a much later phase of maturity than my definition of Stage I oocytes. Naumov's (1956) studies on ovogenesis in the herring also discharge the cyclic appearance of new germinal elements with the comment, "The earliest phases (ovocytes are . . . always present in the ovary." The cells to which he refers, although present at all times in the ovaries of the clupeoid fishes, are oocytes in the process of maturation as shown by his description and figures. They are comparable to Stage III oocytes in the present paper and as such are definitely post mitotic. Under these circumstances, the paper does not provide an answer as to the source of the primitive cells from which the herring ovocytes arise. In addition to the possible sources cited above from which a new crop of eggs may arise, Wheeler (1924) states that the mature ovum is surrounded by a follicle comprised of two distinguishable cell types, and that he feels fairly certain that from one of these types new oocytes will develop. The author is inclined to hold that the cyclic origin of these cells is from the lamellar epithelium, a postulation which gains support from their initial intimacy with the investment. He also purports that only a few cells are derived from this source and that through the intervention of mitosis there is produced the aggregate comprising the nests. His conclusions are open to question for two reasons. First is the enigma of the apparent necessity of the organ to incorporate a number of the sex cells during its origin if it possesses the capacity to develop them later from a tissue type derived from the somatic stromal cells. By following the embryology of the organ, the oocytes are seen not only to be present at all times but their constant replication insures an adequate supply for the first and succeeding crop of eggs. The second aspect of his conclusion that is unique is that the existence of such a condition would represent a major departure of the conservation of stem sex cells in the vertebrates. It is my opinion that these primitive cells are always present in the organ although they may be reduced to such minimal numbers during the sex cycle that they are not observed.

These primitive cells are markedly uniform in size and morph-

ology. They may easily be distinguished from the numerous fibroblasts with which they are usually associated by their greater size and pronounced differences in nuclear structure. While exact measurment of individual cells is difficult due to their indistinct margins, the mean diameter is approximately 7.3 microns and their volume $137^{\cdot 10}$ cubic mm. Spherical forms predominate where they occur singly, but in nests their vague margins often seem to be flattened or slightly concave due to the effect of crowding. A thin complex of delicate connective tissue fibers enclosing an amorphic matrix completely invest the individual cells as well as the nests. Fibroblasts are rarely encountered.

The oocyte cytosome possesses little or no affinity for any of the dyes in the staining techniques used (Plate III, Fig. 9), a condition which has been observed by numerous authors. Particularly pertinent in describing this cellular entity is Hickling's (1935) comment that in the immature hake, *Merluccius merluccius,* the cytoplasm is invisible. In the menhaden it is only in the general region of the nucleus that the cytoplasm retains an almost infinitesimal amount of the acid dyes, but progressively, as the periphery is approached, the substance becomes paler until all color is lost. In the figure the cytosome is represented as the colorless area surrounding the dark nuclear margin. The cytosome is devoid of vacuoles or other inclusions, except that with a magnification of 1450 discrete pale granules can be observed in great numbers. Their number continuously decreases from the nucleus outward. A cell membrane, if present, cannot be seen, although the form of the cell can be closely approximated by the encapsulation of the investing fibers.

Karyosomes are easily recognized. Their diameters are about 4.3 microns and they represent about 68% of the diameter of the cell and about 30% of their volumes. In later stages, as maturation of the oocyte progresses the nuclear diameter and volume will greatly increase but will at the same time become proportionally smaller with respect to the enlarged cell. Structurally it is comprised of a conspicuous karyotheca, circular in form, and a colorless internal matrix. The limiting membrane possesses a thickness of about 0.5 microns and is smooth on its outer face, but somewhat serrate internally. It stains with the basic dyes, thus suggesting a minimal condensation of chromatin on its inner face. In the inter-mitotic phase the internal components of the nucleus consist of a gossamer-like reticulum, the components of which are frequently disposed in a radial pattern, and which is composed of minute granules. The granules are either very faintly basophilic or neutrophilic. A sharply defined nucleolus somewhat less than 1 micron in diameter occupies the center of the nucleus. Its substance has a pronounced affinity for basic dyes for which reason it is opaque.

It is not unusual to find considerable mitotic activity in these cells particularly in young gonads or at the onset in the spring and summer of a new cycle in older ovaries. At such times synchronization of the event seems to occur in all or most of the cells constituting a specific nest. It is characterized by the disappearance of chromatin material from the nuclear membrane and the development of distinct intra-nuclear chromosomes.

Stage II: The transition of the reproductive cells from the Stage I oocytes to Stage II is apparently rapid since intermediate forms are encountered only occasionally. The principal maturational activity occuring in oocytes of this age involves the nucleus within which meiotic activities are in progress. These cells are apparently unable to reproduce mitotically.

Larger or smaller numbers of these cells may be present throughout the year. In adolescent ovaries which are preparing to mature their first crop of eggs a pronounced increase in their numbers occurs during the fall and early winter months. At this time these ovaries are densely packed with such cells and with cells of the earlier stage but contain few or no older stage oocytes. These will not complete oogenesis during the impending spawning season but will attain maturity of Stage III oocytes during the winter and remain in this state until the following season. Plate III, Fig. 10, from an ovary which is about to enter this phase, depicts three cells of this age interspersed among the numerous smaller Stage I oocytes. This condition of partial precocious development postulates that these ovaries, although not capable of completing the cycle of oogenesis within the present season are responding to the same factors which cause the complete maturation of eggs in the older organs. When the tropistic stimuli are changed as with the onset of winter, development will stop until the return of favorable environmental conditions at the following season. The situation in ovaries which are mature and which have produced a previous crop of eggs is considerably different. Here the annual rhythm involving the appearance in large numbers of a new crop of Stage II cells during any seasonal period is confused or absent as shown in Table V. If the data is smoothed by using a moving average of three, it is found that the production of oocytes of this stage in the older ovaries is markedly constant from January to September, thereafter progressively decreasing until December, at which period the numbers present are reduced to about one-half that which existed earlier. The retarded pace of production of Stage II cells in ovaries which have previously matured eggs is due in part to the considerable supply of Stage III oocytes present in the ovary, cells that have been carried over from the preceding spawning season and which will be utilized for the new crop of eggs, and in part due to the fact that in such ovaries there occurs a slow transformation of Stage I cells into Stage II oocytes throughout most of the year.

These cells are seldom found in aggregations characteristic of the youngest oocytes. Those which earlier were contained in the nests begin to migrate to the interior of the ovigerous lamella accompanied and surrounded by varying amounts of connective tissue (Plate III, Fig. 10), while cells originally disposed singly sub-adjacent to the superficial covering of the lamella tend to retain that position. In the latter situation, the cellular enlargement is directed toward the interior of the ovigerous lamella, and such cells carry on their inner face a reflection of the marginal lamellar connective tissues. In this manner their enlargement does not produce elevations of the surface of the lamellae such as occurred in some Stage I oocytes.

Cells comprising this stage are distinguishable by a disproportionally large nucleus, a heavy limiting nuclear membrane, a visible com-

TABLE V

Percentages of oocyte stages by fish and months.

FISH SIZE (MM. F. L.)	MONTH CAUGHT	STAGE I (%)	STAGE II (%)	STAGE III (%)	STAGE IV (%)	STAGE V (%)	STAGE VI (%)
50- 74	August	96	3	1			
75- 99	April	100					
	June	46	18	36			
	August	48	18	34			
	December	8	25	37			
100-124	April	64	15	21			
	June	36	14	50			
	August	35	16	49			
	November			8	92		
	December			77	8	15	
125-149	January			54	23	15	8
	April	36	6	58			
	June	58	5	37			
	August	21	8	71			
	October			100			
	November			82	18		
	December			83	9	8	
150-174	January		12	47	18	18	5
	April	62	12	25	1		
	August	10	6	84			
	October	2	3	75	20		
	December			76	14	10	
175-199	January	10	10	51	23	3	3
	February			42	6	12	40
	April	36	8	56			
	May	36	15	45	4		
	June	2	17	81			
	October	4	8	58	21	5	4
	December			45	18	19	18
200-224	March	7	7	34	33	19	
	April	57	6	37			
	May	25	11	64			
	October			83	17		
	December			47	17	19	17
225-249	August	10	2	81	7		
	December			35	23	18	24

plement of chromatin which in many cells is organized into meiotic chromosomes, the eccentric position of the prominent nucleolus in those cells not in a state of meiosis, and the presence of a distinct cellular membrane. Plate II, Fig. 9, shows three of these cells photographed at the same magnification. The darker oocyte which has been added to the picture from another photograph is slightly more advanced than the remaining two. These oocytes are seldom encountered as true spheres, especially in older ovaries containing many cells in the more mature stages. This crowding effect results in the deeper oocytes assuming pear- or oval-shaped configurations, or, in the case of isolated sub-marginal cells, a flattening along their outer surface.

The mean diameter of late Stage II cells is 22 microns, representing a growth increase of about 350 per cent of the previous stage. The effect of this increment is to materially enlarge their volumes to about 4200 per cent of the Stage I oocytes. Concurrent with cellular enlargement, the nucleus has materially increased its size from 4 microns to about 13 microns in diameter or about 350 per cent, but its comparative volume increase amounts to about 530 per cent.

Specific follicular thecal investments are not present, although the nearby connective tissue fibers are more compactly applied to the surface of the cells than previously. A delicate and moderately well-defined cellular membrane of a neutrophilic character develops as the stage progresses. Cytoplasmic transformations from the previous condition are minor. A continuing increase in its granular content is noted and due to the uniform distribution of the material, the cytosome appears homogeneous throughout. Also, as a result of the greater abundance of this matrix and its enhancement for plasma stains, it appears moderately denser than earlier. Organelles or metaplastic materials were not demonstrated by any of the stains used.

With the onset of Stage II, nuclear enlargement occurs very rapidly so that the body soon comes to occupy most of the cell. Because the cells at this period tend to be more isolated from each other than formerly so that they are subject to less compression, the nucleus assumes a more central location and spherical shape. The enclosed chromatin meanwhile migrates away from the nuclear membrane, and as it does so it rapidly loses its affinity for the basic stains. For a short interval it is neutrophilic but then becomes weakly acidophilic in which state it remains for the duration of this stage. In the interphase condition, the chromatin is distributed throughout the nucleus but because of its fineness and poorly developed tinctorial properties it is indistinct. The inter-phasic character of the oocytes may be observed in at least some cells in all functional ovaries. The state is most pronounced in the few Stage II oocytes that are present during the period of yolk formation and the final maturation of the egg. These cells will lie dormant until the subsequent reproductive cycle. On the other hand in ovaries that are in the process of preparing a new crop of oocytes most of the cells are seen to be in meiosis. At these times the chromatin appears as rather intensely stained threads or chromosomes which are sharply set off against a somewhat colorless background. The material is in this condition in the darker cell in Plate III, Fig. 10. During the early phases of Stage II the nucleolus constitutes a prominent body measuring about 2 microns in diam-

eter and occupying a central nuclear position. It stains intensely with chromatin dyes for a short time after the initial phase of this stage following which it rapidly becomes oxyphilic. Prior to or during the initiation of the meiotic events it moves peripherally to a position against the nuclear membrane. There it may undergo a degree of flattening against the karyotheca and thereafter tends to retain its position throughout the remaining activity of this stage.

Certain significant nuclear bodies first become evident during the mid and final phases of Stage II. At the completion of this stage, their numbers may vary from one to four per cell. The fact that they morphologically and tinctorially resemble the nucleolus has led to considerable confusion, although it is my opinion that their origin and fate is quite different. Speaking of these bodies Wallace (1903), after discussing the role of the germinal vesicle in the formation of oil droplets, stated " . . . that the nucleoli are not transformed either into oil globules or yolk spherules." Similarly, in *Neotoca bilineata* the multiplicity of nucleoli is noted by Mendoza (1939), and in 1940 he reported that "Numerous vacuolated nucleoli may appear within the nucleus." In his description of the nucleus of the yolkless ova of the plaice, Cunningham (1894) recognized that "The nucleus or germinal vesicle is enclosed by a membrane and contains the nucleoli, . . . " and according to Craig-Bennett (1930), the commencement of maturation in *Gasterosteus aculeatus* is described as being marked by the appearance of numerous small nucleoli. Wheeler (1924) also observed the existence of these bodies in the nucleus of the reproductive cells of *Pleuronectes limanda,* which apparently are of universal occurrence in the early stages of oocyte development. Referring to the nuclei of these cells he reported, " . . . they contain irregular masses of material staining deeply with hematoxylin. These masses tend to stay just inside of the nuclear membrane and then form a dark, irregular border with occasional inward projections." The bodies to which he and the other authors cited above refer are comparable to those occurring in the menhaden shown in Plate III, Figs. 11 through 13. Wheeler *(op. cit.)* refrains from identfying them as nucleoli.

These nuclear organelles in *Brevoortia patronus* arise early in Stage II, but their growth and functional activity does not become evident until later. When first observed they appear as minute, acidophilic, irregular masses lying in conjunction with the inner face of the karyotheca. The presubstance involved cannot be identified nor can its origin be stated with certainty. It is possible that the bodies are an elaboration of chromatin material which becomes condensed on the serrate internal surface of the nuclear membrane. No evidence was presented in the material studied that the bodies are true nucleoli nor that they were derived from that body. The early eosinophilic nature of the masses is distinctly different from the basophilia shown by the nucleolus, and their relationship to the nucleolus precludes an intimate relation between the two. Thus, the bodies appear to arise simultaneously in all areas during which period the nucleolus is confined to a local area. In view of their nuclear origin and the interpretation placed by the author on their role in yolk formation, they are hereafter identified as vitello-nucleoli to distinguish them from the single nucleolus whose identity is ultimately lost. Since they are

not at this period actively involved in vitellogenesis, they are appropriately referred to as proto-vitellonucleoli, whereas they will be designated as eu-vitellonucleoli when they participate in yolk production.

Comparative counts of oocytes in various stages of development in ovaries of all ages show a surprisingly small number of Stage II cells relative to the numbers of earlier and later stages. This is indicative that the stage is quite transitory and that the cells quickly pass into Stage III.

Stage III: In adult ovaries, cells of this stage are present throughout the year. As shown in Table V, their relative abundance increases during the fall months preceding winter spawning and reaches a minimum at the completion of the period. In juvenile menhaden that have recently arrived in the estaurine habitat from the offshore spawning grounds, cells of this stage do not occur. In such fish the ovary may contain only the Stage I and/or Stage II oocytes. Nevertheless, during late summer and early fall large numbers of Stage III cells begin to appear in these ovaries.

Oocytes attaining the final phase of Stage III are stable in the sense that they persist in the ovary from one spawning season to the next. This conclusion is verified by the continued presence of such cells during the entire year both in fish that have spawned and in larvae whose sexual development during the period prior to the time spawning is initiated is of insufficient duration to complete the maturation of the oocytes during that season. Furthermore, the absence of extensive atresia of these cells at any period of the year, particularly following the reproductive climacteric, provides conclusive evidence of their retention. The significance of this mechanism is to create an initial reserve supply of partially matured reproductive cells capable of attaining maturity during the early part of the period that the ovaries are becoming gravid, and hence capable of being spawned early during the running period. Since spawning in this species is intermittent, this initial stock will be oviposited first and will be followed by generations of eggs derived from younger oocytes.

The principal diagnostic features of the sex cells at this stage are an intense cytoplasmic basophilia, an increase in number and size of the proto-vitellonucleoli and the initiation of lipogenesis (Plate III, Fig. 12). The inward migration of the oocytes toward the internum of the lamellae which began earlier tends to continue during this stage. Plate II, Fig. 7, taken from an 8 mm December specimen shows many of these dark staining cells most of which are in the deeper areas or centers of the lamellae. This is particularly evident in ovaries which have never spawned. In these instances, the variability in the amplitude of their movements results in some cells being located in the proximity to the surface of the ovigerous lamella, while others are packed throughout the internal stroma. With increase in size of the lamella in more mature ovaries the characteristic internal massing of the cells becomes less obvious and they appear somewhat dispersed among the more mature oocytes.

Considerable growth of the oocytes occurs during this stage, resulting in an increase of their mean diameters at its close to 78 microns, an increase of about 1100 per cent relative to their size at the

beginning of this period. Simultaneously the volume increase comparable to Stage II amounts to approximately 7300 per cent. Cells of this stage are seldom spherical. Typically they show the effects of compression from adjacent cells to a considerably greater degree then observed in Stage II, the forms being roughly cuboidal, trapezoidal, or pyramidal (Plate III, Figs. 11 and 12). Occasional cells occur which are elongated.

A limiting cell membrane cannot be seen, although the intimacy of the investing delicate connective tissue may be erroneously identified as such. The cytosome at the onset of this stage is rapidly transformed from the previous pronounced acidophilic state to a transitory neutrophilia, which is rather rapidly replaced by a weak basophilia during which the cytoplasm is a light blue-grey and translucent (Plate III, Fig. 12), which ultimately culminates in an intense basophilic state marked by a deep blue or purple-black cytoplasm that is more or less opaque (Plate III, Fig. 11, Plate IV, Fig. 17, and Plate VI, Figs. 22 and 23). Evidence that the activating mechanism resulting in the reversal of tinctorial properties of the cytosome is of nuclear origin, is in part based on the immaturity of the follicular theca at this time, which negates its capacity of contributing to the change. Further support for this concept is the contiguousness of the areas of the emergence of the basophilic state at its inception with the nuclear environs. With the onset of the peri-nuclear basophilic state, it appears that material of nuclear origin is being transferred to the contingent cytoplasm. In the affected zones, the basophilic material is in the beginning sharply separated from the more peripheral cytoplasm. These circu-nuclear conformations lie against the outer face of the nuclear wall and assume numerous shapes from a single, unbroken, irregular, encircling mass, to several closely-associated bodies about 0.5 to 1 micron in diameter to finally rough, plaque-like elements. It is assumed that this condition is extremely transitory because of the infrequency of this form of oocyte, and that it is rapidly superseded by a situation in which the substance extends peripherally through the cytoplasm. The substance is characterized by having a greater degree of basophilia than the unaltered areas of the cytoplasm that now occur in a transitory neutrophilic state. This peripheral involvement may be confined to a single area of greater or lesser extent at one side of the cytosome, or it may occur simultaneously from a number of separate regions at the nuclear margin (star burst effect), or again its radial dispersion may occur as an outward extension of a continuous band completely encircling the nucleus. In any case, either irregular, finger-like, frequently-branched extensions may project from the outer nuclear surface in the general direction of the cell margin (Plate III, Fig. 12), or it may give rise to an expanding anastomosing wave enclosing temporarily a few or many areas of unaltered acidophilic or neutrophilic cytoplasm. Because of the great variability inherent to its diffusion through the cytosome, it may be precociously advanced in one or more areas or again it may develop with some regularity throughout. Regardless of these details, the ultimate transformation of the cytoplasmic mass to the basic condition is occasioned by the hypertrophy of its substance so as to completely occupy the oocyte. Culmination of these events occurs during the initial growth of the oocyte and concurrent with the appearance of the first fat globules.

While the role of the nucleus cannot be seriously questioned in precipitating the events described, the exact source and chemical nature of the material may be open to question. It is possible that the initial paranuclear basophilia may consist of ribonucleic acid derived from either the true nucleolus, the proto-vitellonucleoli, or from products released indirectly from the chromosomes. Because of lack of evidence to support the view that chromosomal fractions are involved and because such entities do not normally pass through an intact nuclear membrane into the cytosome, this possibility is subject to further inquiry. The role of the nucleolus is unsupportable because of its earlier disappearance. As regards the role of the proto-vitellonucleoli, a number of factors point to their involvement, including their attainment of a state of maxium development with the appearance of the cytoplasmic condition and their spatial relationship to the points of origin of the basophilic processes. It is assumed therefore that the proto-vitellonucleoli are the source of the regulatory material, which engages the perinuclear cytoplasm by karyothecal passage. It then follows that they are discharging transfer RNA into the areas of the cytosome adjacent to the nuclear wall, thus giving rise to the pattern of localized basophilia described above, which in turn, by diffusion or cytoplasmic extension, is conveyed peripherally. This transfer may exert one or two possible responses in the cytosome, either of which can result in the alteration of the cytosome from a moderately neutrophilic or slightly basophilic condition to an intensely basophilic state. Thus, the nuclear material may become implicated with the Golgi complex, in which case, as has been shown by Gatenby and Woodger (1920) and others, it enchances hypertrophy of this substance and a reversal of the staining properties of the cytosome. The hypertropic manifestations of the Golgi substance in these oocytes would be reflected in an increase of basophilia. Furthermore, since lipogenesis is considered to be linked with Golgi functioning and because the activity is initiated shortly after the development of the basic state of the cytoplasm, it may be concluded that the release of RNA by the proto-vitellonucleoli stimulates Golgi development, which in turn precipitates the development of pronounced basophilia and concurrently introduces the process of lipogenesis. An entirely different interpretation may, however, be formulated to explain the development and intensification of the cytoplasmic basophilia in which it is assumed that the RNA derived from the proto-vitellonucleoli is utilized, as evidence has established, by the ribosomes in the production of RNP. Since these substances react with basic dyes, the appearance and change in the cytoplasmic basophilia may reflect the cumulative activity of the ribosomes. The author is inclined to accept the concept of the role of the Golgi substance in establishing the basophilia because of its accepted function in the formation of lipids, which occurs simultaneously with its appearance, and because ribosomes have been shown to be involved in the production of proteins. The spatial relationship that occurs between the assumption of the basic state and the production of oil is shown in Plate III, Fig. 12, in which the globules are almost exclusively associated with or embedded in the deeper staining basophilic areas. After the attainment of the cytoplasmic basophilia, the possible existence of cytoplasmic inclusions could not be determined by the techniques employed. The duration of this state may be days, weeks, or months since cells in

this condition remain in a dormant state which is known to persist from one to another reproductive cycle.

Regardless of the interim of the basophilic phase, the resumption of activity of the cytosome is manifested by the sudden appearance of a subcortical layer of minute liposomes or fat globules. This development is contingent upon the attainment of the state of extreme basophilia and may or may not be accompanied by a restricted loss of basophilia in the region involved. The initial liposomes are small bodies not more than 1 micron in diameter, spherical and disposed in fundamentally a single layer. Generally, they develop in all areas of the periphery simultaneously, but occasionally they occur in one or more isolated zones. In their primitive condition they appear to be invested by a delicate pellicle which, with Heidenhain's iron hematoxylin and eosin, assumes a more intense basophilia than the adjacent cytoplasm.

Due to the rapidity of lipogenesis, large numbers of the oil bodies are progressively produced internally and concentrically to the original ones. During this activity many of the small liposomes become associated to form aggregates consisting of a relatively small number. At their points of contact with each other, their margins become flattened. It is indicated that shortly thereafter some coalescence occurs. This interpretation is supported by the presence of liposomes of larger sizes, many of which have a number of the smaller types adhering to their surfaces. The production of the additional larger species of fat globules through the union of the smaller forms culminates in the establishment of a circular, sub-cortical band varying in extent from one to four globules aligned along the axes of the cell radii. The average total number observable in a cross section passing through the cell center ranges from 30 to 55, depending on the degree of development of the oocyte. Each globule at this time has a diameter ranging from 2 to 6 microns.

Nuclear enlargement is accelerated throughout this stage, and constitutes a characteristic feature. Lying at the approximate cell center it attains by the close of the period a size of about 51 microns and constitutes 56 per cent of the cell volume. Contrasted to nuclei of Stage II, its volume has increased by approximately 2500 per cent. It is sharply circumscribed by a well-developed intensely basophilic membrane of uniform thickness of about 0.5 micron (Plate III, Figs. 11 and 12). The difference in its appearance in the two figures is in part due to the staining technique employed. Flemming's method was employed in Figure 11 and iron hematoxylin for Figure 12. The membrane is not always easily distinguished from the cytoplasm (Plate III, Fig. 11), particularly in the later phases, because of its similar reaction to dyes. The nuclear lymph is colorless and homogeneous. It supports the protovitellogenic bodies and the chromatin, the latter of which has metamorphosed from the distinct chromosomes frequently encountered in the previous stage to a finely granular, eosinophilic meshwork. This heterochromatin network extends throughout the interior but tends to concentrate slightly near the nuclear center.

Prior to the beginning of Stage III and simultaneous with the first appearance of the proto-vitellonuceloli, the true nucleolus, which

earlier had assumed a position at the nuclear membrane, undergoes hypertrophy, ultimately attaining a diameter between 11 to 13 microns. In this state it is easily distinguishable from the proto-vitellonucleoli. Concurrent with and subsequent to its enlargement it manifests a progressive loss of affinity for the basic stains. In many preparations it assumes hyaline characteristics and a translucent blueness, and its outline becomes more and more indistinct. These reabsorptive processes appear to continue until it becomes completely dispersed.

With the disappearance of the nucleolus, nuclear synthesis of considerable numbers of proto-vitellonucleoli occurs. The earliest generation appears to mature from the granular elements which are disposed around the inner karyothecal surface and which are believed to have been present as minute eosinophilic particles in the previous stage. These progressively enlarge *in situ,* meanwhile momentarily becoming neutrophilic and ultimately, intensely basophilic. While these changes are in progress at the margin of the nucleus, additional generations of the bodies appear to arise from the substance of the eosinophilic granular reticulum in the internum of the nucleus. At numerous points in this complex, granules occur which are slightly larger than those constituting the reticulum proper and which at first react identically with the same dyes as the reticular material. With further enlargement they round up and their peripheral limits become sharply defined. One or two such inclusions are visible in the darkest oocyte in Plate III, Fig 11. The centrally located granules are at this period from 0.2 to 0.4 microns in diameter. A short interval intervenes during which they develop a more intense eosinophilia before a transitory neutrophilia takes over. This state is followed by a further increase in size and the appearance of basophilic characteristics, culminating in the attainment of a tinctorial status identical with the sub-karyothecal bodies. Because of temporal differences which are involved in the origin of these bodies, there is present in the typical Stage III nucleus from 15 to 30 or more of the minute developmental stages, a smaller number of intermediate forms and relatively few of the final stages which have a diameter between 7 and 8 microns. In the terminal phase of this stage approximately 90 per cent of these supplemental proto-vitellonuclei of internal reticular origin are definitely basophilic, and the formative activity continues at a progressively decreasing rate into the following stage.

Although during the period of inception and growth of these proto-vitellonucleoli they are dispersed in the reticulated nuclear complex, there seems to occur a progressive, uncoordinated movement of the larger forms toward the nuclear wall in such a manner that some have not reached the nuclear membrane, others have merely engaged the membrane, while still others are in various stages of affixing themselves to the karyotheca during which their outer face becomes flattened and tends to conform to its curvature. These are now indistinguishable from the earlier type which arose directly in conjunction with the nuclear wall. These developments collectively have resulted in the application to the karyotheca of a more or less uninterrupted investment of these elements around its internal face.

There are reasons to assume that functionally the proto-vitello-

nucleoli of peripheral and internal origin are identical. They are morphologically and tinctorially indistinguishable in their final state; both pass through a stereotyped metamorphosis, involving only temporal and spatial differences, and no selectivity is involved in their ultimate distribution on the karyothecal surface. Their synthesis, mediated by the chromatin, could readily be accomplished at any area within the nucleus since this material is widely distributed at this period.

Stage IV: The principal distinguishing characteristics of Stage IV oocytes are a loss of cytoplasmic basophilia, the appearance of small provisional yolk granules, a relatively minor increase in cell size, a beginning regression of the nuclear wall, the transformation of proto-vitellonucleoli into eu-vitellonucleoli, and the reassumption of a spheroid state. These cells are never encountered in immature ovaries during any season of the year nor are they present in mature gonads except in preparation of the spawning season, during that interval, or for an abbreviated period immediately thereafter. Unlike the retention of oocytes in the previous stage, oocytes in Stage IV which fail to reach maturity and ovulate by the time the annual spawning period is complete are reabsorbed. In *B. patronus* during the interim from January to March they constitute more than a fourth of all oocytes in the ovary (Table V). The maximum frequency of 38 per cent occurs in February, whereas in April their numbers represent about 12 per cent of oocytes present. Except for a rare inclusion of an isolated one during the late spring and early summer, they do not appear again until August. During this month they amount to 4 per cent of all stages, increasing thereafter to 5 per cent in October, 9 per cent in November and 13 per cent in December. Those present from December to March apparently reach maturity during the current winter spawning season, although under favorable conditions resulting in a prolongation of the running period a few present in April may also complete their maturation and ovulate. Considerable atresia of this stage oocyte is observed in April and May, indicating a prior termination of the annual period of oogenesis.

The ovary presents evidence that Stage IV is quite transitory and that little time is required for these cells to complete the transformation from Stage III to Stage V. This is borne out in part by the disproportionally small number of these cells in the gonad during fall and early winter, a condition from which it is assumed that a reverse of these forms is not established considerably in advance of final maturation, but that they continuously progress from Stage III to Stage V. Further substantiation is based on the degrees of development within the stage. The cells in this stage, unlike Stage III, do not evidence a modal point in which they remain in a stage of *status quo* throughout the year.

These oocytes occupy all possible positions in the ovigerous lamellae, being disposed at random with respect to the younger and older oocytes present. The youngest of these cells are reminiscent of the irregular forms characteristic of the previous stage, but prior to entering the subsequent stage they assume a more rotund appearance. The rounding up of the oocytes is due to a greater degree of isolation of the cells, less plasticity of the cytosome, and to the greater rigidity of the more highly developed follicle.

Terminal Stage IV oocytes attain an average diameter of about 0.15 mm, an increment of 190 per cent of Stage III, while their volume has enlarged by approximately 400 per cent. This rate of increase is much less than that occurring in the younger stages.

The primordium of the vitelline membrane is thought to be present as an eosinophilic investment, approximately 0.3 microns thick, constituted of a dense, finely granular material with acidophilic properties lying at the surface of the cortical cytoplasm and not sharply separated from the cellular substance.

Events within the cytosome at the onset of this stage are characterized by a reversal of its staining reaction from the prior basophilia to a pronounced eosinophilia. This transformation in the majority of oocytes seems to occur initially in the subcortical areas (Plate IV, Fig. 16) from which it spreads irregularly toward the cell center. It poses numerous variations in the manner of its origin. Thus, it may be initiated at more than one position with subsequent cortical coalescence of the several areas or it may develop simultaneously throughout the entire marginal zone. The inward face of this oxyphilic cytoplasm is markedly ragged in nature and is often associated with the development of finger- or root-like processes which branch and metastisize in its inward extension into the basophilic material. This may so occur as to establish an encircling, finely granular, acidophilic reticulum, the elements of which surround and enclose variously sized areas of the basophilic substance. These detached basophilic entities soon assume the same form of eosinophilia as the invading reticulum. By the continuation of this process the whole of the cytosome is soon converted to the acidophilic condition. After these changes have been accomplished, the cytoplasm assumes a homogeneous, finely granular, acidophilic condition which, however, is altered by the development of a few or numerous colorless crevices of unknown significance and which may be artifacts. These may be simple or branched, of a greater or lesser length, and disposed in various directions with the majority, however, conforming roughly with the cell radii. The presence of these crevices, together with a slight loss of affinity for stains, imparts to the cytosome a washed-out appearance. These changes usually occur during the later phases of this stage and may constitute areas of cytoplasmic streaming.

The rate of oil production diminishes during this stage. Globules produced previously increase in size either by coalescence or by supplemental lipogenesis. Since only a minimal number of small, new liposomes are established, the cell as it approaches the final phases presents a circumferential band of prominent lipoid bodies irregularly arranged and from one to two in depth as shown in Plate III, Fig. 13.

The production of provisional yolk granules is instituted following the loss of cytoplasmic basophilia. Morphologically this form of yolk is distinguishable from the so-called cubical yolk which appears subsequently and has been designated by Craig-Bennett (1930) as "reticular yolk." In the large scale menhaden the synthesis of the earlier type yolk directly involves incipient material present in the oocyte nucleus as well as specific formation elements occuring in the cytoplasm. Although the literature on yolk production is extensive and assigns to various cytoplasmic organelles the role of its formation,

direct nuclear participation has been both affirmed and disclaimed. In many fish the so-called yolk nucleus has repeatedly been implicated with deutogenesis. Thus, in *Zoarces* a cytoplasmic complex identified as the yolk nucleus is considered by Wallace (1903) as the point of origin of these granules. In *Zoarces, Syngnathus,* and *Zeus faber* he describes the yolk nucleus to have " . . . the form of a cap of deeply staining substance directly applied to one side of the germinal vesicle . . . " which he states later breaks up into small pieces in the cytoplasm. Similar nuclei have been observed by Cunningham (1898) in the flounder, the pipefish, in which he states there may exist from three to four, and in the Pleuronectidae. A few specimens of menhaden ovaries used by the author exhibited similar, more or less compact bodies, but in each instance the organs were obtained from fish that were preserved whole prior to the ovariectomy which suggests that their appearance may result from poor fixation. Wheeler (1924) has commented on the significance of the yolk nucleus in oocytes of *Pleuronectes limanda,* which he concludes is an "inactive body; at least it does not contribute visibly to the formation of yolk . . . ," adding the observation that it finally diminishes and disappears. With further reference to the possible progenitor of the yolk substance, he calls attention to Wallace's *(op. cit.)* observation that the yolk nucleus as usually described is the centrosphere and that the body described by Franz also corresponds to the centro-sphere of Wallace. Regarding the concept of centrosphere contribution in deutogenesis, it has been assumed by Gatenby and Woodger (1920) that the centrosomal milieu, the archoplasm, is involved, and by Meves (c. f. Bolognari, 1958) that the idiosomal substance is the progenitor of the yolk. Centrospheres and associated cytoplasmic components should, however, be held suspect since they are characteristically fiber forming bodies. Possible additional cytoplasmic deutogenic organelles are also implicated by Wheeler (1924) in his statement, "It seems probable that yolk formation is intimately connected with inclusions of the cytoplasm known as the Golgi apparatus and mitochondria." In partial support of this conclusion, Malone and Hisaoka (1961) contend that the yolk of the zebra fish, *Brachydario rerio,* is of two types, i. e. extra-vascular and intra-vascular, and that the former appears to be derived from mitochondria. The synthesizing agent of the latter could not be established. On the other hand, developing yolk globules have been described as being encased with a thin sheath of argentophilic material leading to the interpretation that they are formed at the surface of the Golgi complex. Bolognari (1958) has noted this condition, which he states is discernible with the light microscope, but does not correspond to the ultra structure demonstrated by the E/M. He holds that vitellogenesis is a function of submicroscopic filaments constituting a portion of the ergastoplasm. Since these components were shown to contain ribonuclear proteins, they may consist of or be comparable to ribosomes.

In *Brevoortia patronus,* yolk formation is not only under the control of the nucleus, but particulate, karyothecal material is actually utilized in some manner in the process. After the oocyte cytoplasm has attained its oxyphilic status, the proto-vitellonucleoli lying at the periphery have increased in numbers to the extent that they become crowded and more or less confluent (Plate III, Fig. 13), thus establish-

ing a peripheral ring, which is rarely interrupted in its course. The circular, composite entity now appears after iron hematoxylin as a non-transparent, purple-black, compact investment, intimately affixed to the nuclear membrane but with a clearly defined internal face. While these events are in progress and continuing for a period afterward, there begins to appear on the cytoplasmic surface of the nuclear membrane at restricted or more extensive loci one or several rudimentary deposits of basophilic material. When first observed, their outer surfaces are ragged and sharply delineated from the adjacent cytoplasm. The nuclear face of this cytoplasmic material is for the most part smooth and follows the curvature of the nucleus in such a manner that separation of the intra- and extra-nuclear substance is sometimes impossible. Shortly after the initial appearance of the extranuclear basophilia, it spreads rapidly around the nuclear periphery and by coalescence or otherwise gives rise to a continuous, somewhat serrate investment. The original thickness of this layer usually varies from 1 to 3 microns, although not infrequently it is much more precocious at one side of the nucleus than elsewhere. Repeated observations of the sections established the existence of local continuities between the intra-nuclear condensation and the material on exterior of the nuclear membrane during the initial elaboration of the peri-nuclear material. Later, due to the increase in densities and abundance of the materials, the phenomena become obscured. Where these small, extra-nuclear granules or masses occur, it is occasionally possible to observe a tenuous aspect or a discontinuity of the karyotheca and a direct transfer of material which constitutes the protovitellonucleoli to the adjacent cytoplasm. With the institution of this exchange, the discharged nuclear components become identified as the eu-vitellonucleoli by reason of their extra-nuclear position and their impending role in yolk formation. It is not known what factors are involved in the regression of lysis of the nuclear membrane at the points where proto-vitellonucleoli are transformed into eu-vitellonucleoli. With the progression of the stage, the occurrence of the eu-vitellonucleoli masses increases.

The significance of these observations is to establish that in menhaden oocytes a direct transfer of material of nuclear origin to the cytoplasm begins immediately prior to the appearance of yolk. This phenomenon either is not involved in the majority of fish or it has been overlooked, as indicated by a review of the literature. The only instance involving fish oocytes known to the author where the matter has received comment is in the case of *Pleuronectes limanda.* Of the nuclei of these cells Wheeler (1924) reports that "They contain irregular masses of material staining deeply with haematoxylin. These masses tend to stay *just inside* the nuclear membrane and thus form a dark, irregular border with occasional inward projections." (Italics are those of the present author.) However, the question of the ability of a nucleus to extrude particulate material has been established in other organisms. Thus, Wheeler *(op. cit.)* relates that in the annelid *Sarccocirrus,* Hemplemann observed the nucleolus to break up and its fragments to pass into the plasma thus marking the areas at which yolk production is initiated. This observation had been earlier affirmed by Gatenby (1922), who concluded that in the same annelid the "nucleolar deutoplasm" is produced from extruded ma-

terial of the nucleolus. Further evidence of nucleolar fragmentation and subsequential discharge of its fractions into the cytoplasm as progenitors of yolk has, according to Wheeler, been observed in *Lumbricus* by Calkins. These views are discounted, however, by Wheeler (1924) in *Pleuronectes limanda* in his statement, "There is no evidence in the dab of the liberation of nuclear pockets, each containing a nucleolus." Cunningham (1898) called attention to observations of Schraff that vitelline elements are derived from nuclear substance, with which Cunningham did not agree. That the nuclear proto-vitellonucleoli are the precursors of the cytoplasmic mechanism involved in yolk formation in the menhaden must be postulated from the present study. As such they may act directly as foci of yolk production or contribute to the organization of the Golgi substance, which is believed by Wheeler (1924) to be directly involved, and which present day knowledge would tend to rule out, or again they may become associated with the development and functioning of the mitochondria or ribosomes. The role of the mitochondria has been examined by Malone and Hisaoka (1961) in the case of the zebra fish, in which they found that two forms of yolk appeared, one of which was derived from mitochondria and was called extra-vesicular. It is not within the scope of the present study to establish which of the cytoplasmic organelles are involved in yolk production although it is thought that the ribosomes may well be implicated.

While nuclear extrusion of the proto-vitellonucleoli is still in progress, those which initially entered the cytoplasmic zone as eu-vitellonucleoli become disorganized and their substance disassociates locally into the cytoplasm resulting in a reversal of its tinctorial properties from oxyphilic to basophilic. This transformation, which originally occurs in the immediate vicinity of the nucleus, is active throughout most of the remaining part of this stage. Through supplemental augmentation of eu-vitellonucleoli material the zone is progressively extended toward the cortex of the cell. Its outer margins appear in the form of tufts, roots, or streamers (Plate III, Fig. 13). During this peripheral advance, the distal extents of the processes begin to lose their basophilia and assume a strong affinity for the acid dyes. Along their courses, at the points where changes in the staining properties are occurring the transitional areas show a momentary neutrophilia. Although these terminal arborizations and the cytoplasmic background in which they lie are both acidophilic at this time, the morphology of the processes is quite evident because of their greater affinity for the dyes. This alteration of staining properties may not occur uniformly in all parts of the cell, but in any area where it is instituted, a progressive wave of neutrophilia followed by acidophilia advances inward. These events culminate rather early during this stage in the complete dissolution of the basophilic complex, at which time the nucleus also appears devoid of the marginal massive form of proto-vitellonucleoli.

A recapitulation of alternations that are presented in the staining properties of the oocyte cytoplasm up to this period shows a chromophobia at Stage I; a weak to moderate acidophilia at Stage II; an initial slight basophilia followed by intense basophilia (associated with the onset of oil production) at Stage III; a transitory return to an acidophilic condition (similar in degree to Stage II) at the begin-

ning of Stage IV, succeeded by a radial outburst from the nuclear margin of basophilia that is replaced by a rather strong acidophilia (presaging the period of yolk production). It is significant in gaining an insight into the cytoplasmic synthetic activities of the cells that basophilia developed at only two intervals each of which immediately preceded the appearance of metaplasmic components, i. e. oil and yolk. Furthermore it has been shown that the basophilia arose at the nuclear surface thus invoking a transfer of nuclear regulatory substances to the cytoplasm.

From the inception of the cytosomal extensions of the basophilic processes, there appear along their inter-faces great numbers of sharply delineated neutrophilic and eosinophilic granules which, when first evident, are between the limits of visibility and 1 micron. The earliest of these granules are discernible in the environs of the nucleus, after which their appearance is correlated with the outward growth of the basophilic projections. These are recognized as the provisional yolk granules. While the first generations are increasing in size, sequential generations are contributing to an augmented supply. During Stage IV, the provisional yolk granules attain a diameter of a little more than a micron and retain their neutrophilic or eosinophilic character. As the oocyte passes into the following stage, several thousands of these granules have been formed.

The centrally-located nucleus is fundamentally spherical. It is sharply defined by reason of its membrane and the condensation of the deutogenic bodies. In the final phases its diameter is about 37.5 microns and its volume equivalent to 16 per cent of the cell. Its size is also significantly indicated in that its volume has increased by approximately 1100 per cent relative to Stage III nuclei. Thus while it continues to increase somewhat in size, its rate of enlargement is superseded by the rate of cytoplasmic growth. The nuclear enlargement is correlated with the initiation of yolk formation, and constitutes the final material increase in its size to occur during maturation of the egg.

Most of the details of nuclear morphology and function have already been described in connection with its role in the production of the cytoplasmic yolk. Its internum is constituted, as in the previous stage, of a finely granular material arranged in a delicate network suspended in a chromophobic nuclear sap. Basi-chromatin or discrete chromosomes are never present.

Stage V: The activities of oocytes at this developmental stage involve primarily the maturation of the deutogenic elements and the initiation of a second phase of lipogenesis. In fish of less than 100 mm fork length this stage does not occur during any month of the year. In larger females these oocytes are present in the ovary from October (this observation being based on a single specimen which contained a few in the early phase of this stage) to March inclusive, although none were present in two specimens taken in November. Their numbers reached a peak in February. During March and April a residual number of oocytes of this stage are also present, most of them evidencing some degree of atresia, as is likewise the case of an occasional one in February. In such cases, the temporary retention of

the oocytes is occasioned by the termination of the spawning season prior to their complete maturation.

The duration of this stage could not be accurately established, although it was evident that the period shows marked variability with respect to individual ovaries and single oocytes. In view of their frequencies during the seasons given in the account above, those that begin this phase of maturation during the late summer or early fall show a leisurely progression of development, as compared to an accelerated rate for the crop of cells entering this stage at the approachment of the spawning period. Under such circumstances, individual oocytes are thought to remain at this maturation level for a period of a week or two as to as long as a month or more. As a corollary, oocytes remaining in Stage V for the longer period originate from the crop of Stage III and IV cells which were present in August and September, whereas the abbreviated condition is associated with the rapid transformation of Stages I and II cells into the Stage V oocyte in the interval between summer and the onset of spawning.

These oocytes are disposed throughout the interior of the ovigerous lamellae. The most significant cytological features of these cells include a material increase in cytoplasmic volume, an intensification of nuclear oxyphilia, the appearance of nuclear degenerative activities, the maturation of the yolk bodies, and the addition of a second generation of fat globules (Plate IV, Fig. 14). The mean diameter of the cytosome is in the order of 0.36 mm and its volume has increased by 1300 per cent from the previous stage. The growth of the oocyte is accompanied by a comparable enlargement of the nucleus so that ratio of nuclear to cell size remains as one to four which relationship also existed in oocytes of the previous stage. Oocytes at this time are markedly spheroidal and are surrounded by a well-developed follicle which is described beyond.

The final events pertaining to fat production in the egg occur at this period. During most of this stage fat globules produced earlier in the peripheral areas of the oocyte have greatly increased in size by interstitial enlargement or by coalescence, or both, so that forms of varying sizes lie more or less irregularly contiguous with each other, both radially and circumferentially so thus imparting a frothy appearance to the region. During their enlargement the more internally-located sub-cortical globules begin to migrate toward the deeper parts of the cytoplasm and assume a position in the environs of the nucleus leaving a residual number in the cortical layer which persist until ovulation. Coincidental with this inward migration there suddenly appear considerable numbers of minute, newly-organized globlues at the periphery of the nucleus. The youngest of these liposomes are from 1 to 2 microns in diameter, colorless, and not surrounded by a visible limiting membrane. Because of the intimacy of the nucleoplasm and the cytoplasm at their points of origin, the source of the formative material could not be established. A rapid growth increment soon transforms the original minute vesicles into an encircling layer at the nuclear margin. Their mean diameter increases to about 8 microns during this organizational phase, but by further growth and coalescence a considerable number will essentially double their size by the time the oocyte passes into the next stage. In the process of

establishing these circum-nuclear aggregates through coalescence, many of the components are seen to consist of a central globule which bears on its surfaces a single layer of globules of equal or smaller size in the pattern of a single-layered rosette. In this condition, delicate pellicles become evident at the interfaces. Single layered rosettes are progressively transformed into types possessing two or more rows of peripheral globules surrounding the central lipid body. Sequentially the centrum of the globule shows further enlargement by the contribution of oil from those lying on its margin. During most of this period, many strands of nucleoplasm extend outward between the encircling globules and establish a continuity with the cytoplasm (Plate IV, Fig. 15).

The source and manner of production of fat during oogenesis of the fish egg is in need of further clarification. Although it has been established by numerous investigations that the quantity and organization of the material in these oocytes follows no consistent pattern between the different major groups, and in some cases between closely related species, it would appear that the underlying mechanism for its production should evidence some predictable consistency. Wallace (1903) has tentatively considered that the yolk nucleus may, at least in some cases, be the locus of fat synthesis. He associates the disappearance of this body with the simultaneous appearance of oil in the oocytes of a number of fishes, and he contrasts this condition with other fish such as the plaice and dab, whose eggs do not contain the lipid bodies and which do not develop the so-called yolk nucleus. The mechanism of oil production by a body considered by numerous investigators to be specifically organized for yolk formation is interpreted by Wallace to involve a form of protein degradation in which oil is an end product. He cites evidence supporting this concept by calling attention to the appearance of fatty degeneration products derived from yolk bodies in atretic eggs. He does not infer, however, that oil must be derived from yolk material, but proposes that a protein anlage must exist whether it be within the yolk nucleus or in the yolk itself. However, due to the absence of a recognizable yolk nucleus in menhaden oocytes, this body must be disregarded as an oil producing mechanism. Wheeler (1924) gives an excellent description of the role of the Golgi material in the production of yolk but eludes the problem of lipogenesis. Regardless of the point of origin of the oil in the oocyte, whether peri-nuclear, adjacent to or within the yolk nucleus (which Cunningham (1898) has identified as being the centrosphere), or from visibly unorganized regions of the cytoplasm (as occurs in the menhaden during the early phase of the homogeneously basophilic yolkless oocyte), and which condition has also been referred to by Wheeler (1924), Wallace (1903), and Cunningham (1898), substantial evidence compiled from many studies involving an array of different material tends to affirm that the formative apparatus is the Golgi substance. If this is the case, Wheeler's conclusions are apropos when he states that the oil producing substance can be demonstrated during different phases of the growth cycle and that whole series of images exist. It may thus be incorporated in the so-called yolk nucleus or be diffused throughout the cytoplasmic matrix. If this is the case, fat may appear in the various forms of oocytes at diverse locations in the cells, according to the conformation of the

generative material.

Yolk is both produced *de novo* and matured at this time so that the oocyte passing into the final stage has essentially its full complement of the material. The appearance and types of yolk bodies have been variously described by investigators. Naumov (1956) recognizes two forms, a small type which he describes as oval rounded and characterized by staining poorly with Heidenhain's hematoxylin, and an irregularly cuboid type which he calls cubical yolk and which stains intensely. He does not make clear whether one of the types is a developmental stage of the other. Menhaden yolk is constituted of large and small globules, the former of which in its development passes through a somewhat cubical condition as in herring oocytes. Numerous intergrades exist between the large and small bodies, and their separation in the menhaden into inner and outer zones is not pronounced as has been indicated by Naumov (1956) in herring when he described "a small layer with round-oval yolk globules" lying adjacent to the nucleus and that " . . . then follows cubical yolk, occupying nearly the whole oocyte." The smaller yolk bodies in the menhaden oocyte appear to develop directly from the small eosinophilic and neutrophilic granules which arose in Stage IV. These and the additional generations which are produced in the same manner in the present stage are not associated with pellicles or a vacuole, but after attaining a size of about 2 to 3 microns develop an intense basophilia. Thereafter they do not increase in size.

The larger or cubical type of yolk arises more distal to the nucleus. The mode of its production is best observed early in this stage, at which time the details are not obscured by a preponderance of yolk. In some preparations at this period, many rather dense, flame-like strands of eosinophilic material which originated during the prior stage can be distinguished from the weaker staining cytoplasm. Along the margins of this material large numbers of minute, basophilic yolk bodies suddenly appear, each of which is enclosed in a delicate pellicle which is separated from the enclosed yolk granule by a chromophobic refractive investment or a small space. Staining of the yolk material at this time is not uniform so that it often presents a moderately light hyaline internum which is surrounded by a cortical zone of greater density. In other cases variable sized sectors are frequently observed that stain more intensely than the intervening segments. While the yolk granules are in this state they continue to increase in size and staining intensity. Having attained a diameter of about 3 microns, the yolk bodies still enclosed in their investing pellicle appear to move away from their points of origin into areas of the cytosomes not occupied by the deutogenic strands. At its point of formation there remains a semi-circular lacuna whose smooth surface is of the size and contour of the pellicle. Since many of these bay-like depressions can be found along the faces of the deutoplastic strands, they import to them a distinctly serrate appearance. As the process of yolk production continues, the repeated erosion of the radially oriented deutogenic strands frequently causes a complete severance of their substance at one or more points, thus giving rise to the irregular shaped islands of various sizes that are distinguished by an encircling marginal presence of the lacuna between which narrow frayed filaments of the eosinophilic matrix project a short distance out-

ward. Each of these isolated island fragments continues to carry on yolk formation in the manner described until the yolk producing matrix is completely consumed.

As the yolk granules further mature, their diameters increase to about 5 microns and they tend to stain uniformly in contrast to the heterogeneous condition present earlier. The investing pellicles become a little thicker and more distinct. Sooner or later during this and the following stage, each enclosed yolk body begins to show evidences of regional achromia. When this occurs the basophilic yolk body appears to fragment *in situ* into from two to five or six portions by the appearance of narrow achromatic crevices whose margins are smooth and which may be oriented as radii, chords, or combinations of the two. The particles thus produced continue to remain together as a single entity. This represents the cubical condition of yolk as it occurs in the menhaden. The degree of chromasia is progressively reduced as the achromic interfaces or crevices slightly enlarge, and ultimately all the fragments within each aggregate assume a slightly acidophilic state. These terminal changes may occur in a relatively small proportion of yolk granules in Stage V, although the greater part of the activity is delayed until later. The pellicle is retained during most of the period of transformation, but with its ultimate disappearance the yolk granule occurs in a clear cavity not sharply delineated from the granular cytoplasm.

Because of the peri-nuclear encroachment of oil globules from the outer cytoplasmic areas, the outpouring of the eosinophilic nucleoplasm is channeled between the margins of the prominent fat masses (Plate IV, Fig. 15). Its exodus is thus in the form of radial strands that are limited marginally by the fat bodies. Morphologically or tinctorially no distinct line of demarcation exists between nuclear and cytoplasmic extents although it imperceptibly shows less chromasia as it continues into the cytosome. Finally the material becomes indistinguishable from the ground substance of the cytoplasm. As if swept along by the discharge of the nucleoplasm, there occurs an outward movement of great numbers of small, basophilic, spherical granules. The particles were first visible in Stage IV (Plate IV, Fig. 13). Unlike the proto-vitellonucleoli they always remain free in the nuclear sap rather than being condensed on its membrane. They are also different in the respect that when first seen they have an affinity for the acid dyes (which was not the case with the proto-vitellonucleoli), following which they increase in size and become basophilic. At the time they escape into the cytoplasm they have a diameter of approximately 1.3 to 1.6 microns. Shortly after their appearance in the nuclear matrix a clear area or pellicle arise on their periphery. This enlarges slightly as they move out of the nucleus and continues to adhere to the yolk material as it is transported into the cytoplasm.

Upon entering the cytosome, the nuclear bodies become indistinguishable from the older yolk granules with which they commingle. This phenomenon points to the probability that the pre-substance of yolk is produced in the karyosome as regards both the earlier and the final forms. In the first case the process involved the streaming or escape through a sieve-like nuclear membrane of a basophilic pre-sub-

stance derived from the proto-vitellonucleoli, and in the second instance a direct discharge of the same or comparable material into the cytoplasm.

The final dissolution of the nucleus occurs when all of its substance has been liberated into the cytoplasm. During this terminal period the space formerly occupied by the body becomes progressively given over to yolk until ultimately no distinction can be made between the material that earlier was present between the karyotheca and the cortical zone of the oocyte, and the new yolk that occupies the area where the nucleus resided.

The so-called lamp brush chromosomes are present in the nuclei throughout most of Stage V. They disappear after partial regression of the nucleus has occurred. In the menhaden oocyte, the numbers observed ranged from none to eight per nucleus. They are of diverse lengths some being in the order of 12 or more microns in length but their widths were uniformly about 0.2 microns thick. Characteristically, they appear in a serpentine or loosely coiled pattern with vague margins and are not vividly distinguishable from the ground substance. Cajal's method was most effective in their demonstration.

Stage VI: Oocytes which have completed the maturation events of this stage are in a condition for spawning. From the data available (Table V) it is therefore concluded that spawning occurs in the localities from which collections were made between the latter part of October to March. During this interval the relative numbers of mature cells in the ovaries increased from about 4 per cent in October to a mode of 40 per cent in February. The spawning pattern of *B. patronus,* while total in the sense that it is completed during a single season, occurs at frequent intervals during the running period. This postulation is based upon the observation that the ovarian ova at this season are comprised of a composite of spawnable eggs, eggs which are almost ready to ovulate, eggs in Stage V which will complete their maturity prior to the cessation of spawning, and a number of Stage IV oocytes of which many are thought to mature before spawning is complete. Intermittent spawning is essential in this fish since the number of eggs released is of such magnitude that they could not be contained within the ovary if they reached maturity simultaneously. Apparently the pattern occurs rather widely among the clupeids. Naumov (1956) has reported the condition in the herring, and in the case of the Atlantic menhaden similar conclusions were given by Reintjes (1961).

During this stage all nuclear and cytoplasmic changes are terminated in the final transformation of the oocyte into an ovum. The events that characterize oocytes of this stage are the absence of a visible nucleus, the occurrence of a peripheral layer and a central mass of lipoid globules, and the presence of massive numbers of mature yolk bodies (Plate V, Fig. 16).

The final stage oocyte is spheroidal with a diameter of slightly more than 0.5 mm in paraffin sectioned material, thus constituting a diameter increment of 134 per cent of Stage V oocytes. The cell volume has simultaneously enlarged by 241 per cent. This represents the smallest increment change in diameter and volume between suc-

cessive stages that occurs during oogenesis.

In the final stage the cell is devoid of a recognizable nucleus and consists entirely of cytoplasm and its inclusions. Yolk material is distributed throughout the cytosomes, except in areas occupied by the oil globules and in the thin cortical zone. Yolk particles in the subcortical regions are large and occur in such a great abundance that they lie in contact with each other so that little or no cytoplasm is visible. The more internal elements, by reason of their lesser number and size, are more or less isolated from neighboring entities by irregular reticulated strands of faintly acidophilic hyaloplasm. Irrespective of size or position of the yolk granules, each continues to be surrounded by the clear pellicle present in Stage V oocytes.

According to their tinctorial response, the deutoplasmic components fall into two general categories. One type manifests a moderate polychromasia wherein the affinity for acid dyes is dominant. After Heidenhain and phloxine, their color deviates from red or lavender to deeper tones of reddish-purple; after Flemming they are generally red but occasionally may incorporate small areas of violet, and after Cajal they display different intensities of green. This type of yolk granule is structureless internally. A second group develops a strong affinity for the basic dyes, appearing deep purple or purple-black after Heidenhain, purple after Flemming, and blue after Cajal. This group of bodies is not only distinguishable by the individual colors, but their substance sequently appears to fragment within the encompassing pellicle into from two to ten fractions whose varied sizes and shapes bear a likeness to the pieces of broken discs. This change is followed by the development of a marginal chromophobia that directly progresses to the internum. When the loss of color is complete, the yolk granule appears to have been removed. Plate V, Fig 16, shows a generalized view of the appearance of the yolk after the loss of an affinity for stains. Lack of synchronization of the development of the achromia in the individual fragments comprising a single yolk granule is a frequent occurrence. There remains for a variable period following the attainment of the achromatic condition a finely granular, almost colorless sphere of the dimensions of the original vitelline body. The faint vacuolar halo persists although the pellicle could not be identified in the final phase of this maturation process. With the techniques employed, the achromatic body could not be readily distinguished from the minimal quantity of adjacent cytoplasm because of their similarities in staining and because definite margination of the yolk body no longer existed.

Other vitelline bodies lose their tinctorial properties in a more direct manner, in which case their deep basophilic substance does not fractionate but, by a generalized loss of staining affinity initiated simultaneously at all areas of the yolk periphery and progressing uniformly toward the internum, is progressively reduced until it disappears. The remaining terminal stage of the achromatic vitelline element thus derived is enclosed in a colorless halo (without visible pellicle), and cannot be distinguished from those previously described.

Regardless of which of the above methods are involved in the final maturation of the yolk bodies, their terminal condition is identical. Since large numbers of separate colorless yolk granules were

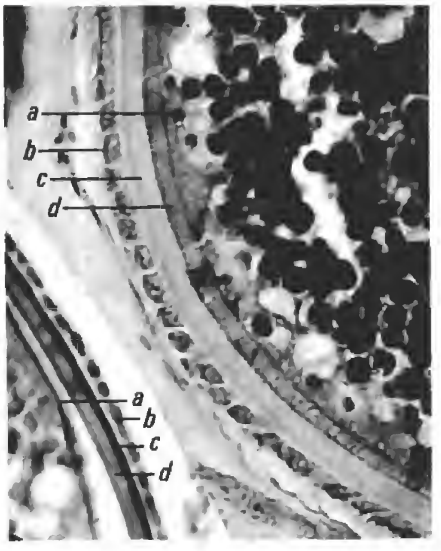


Fig. 18

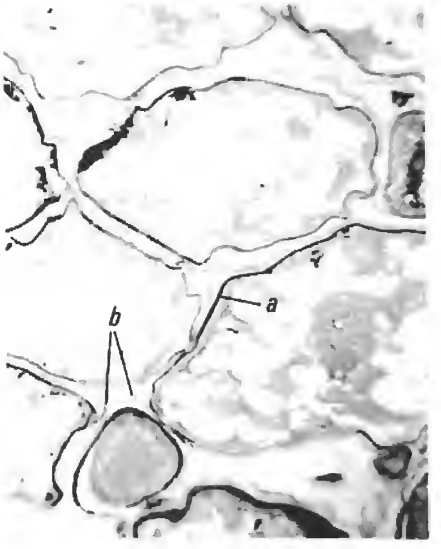


Fig. 19

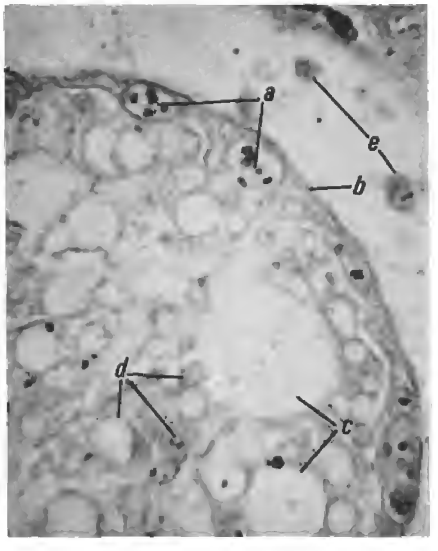


Fig. 20

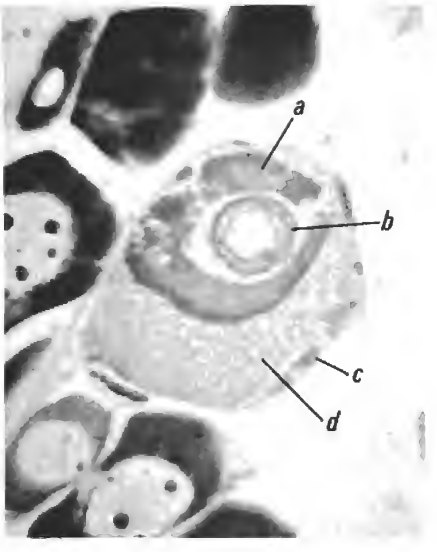
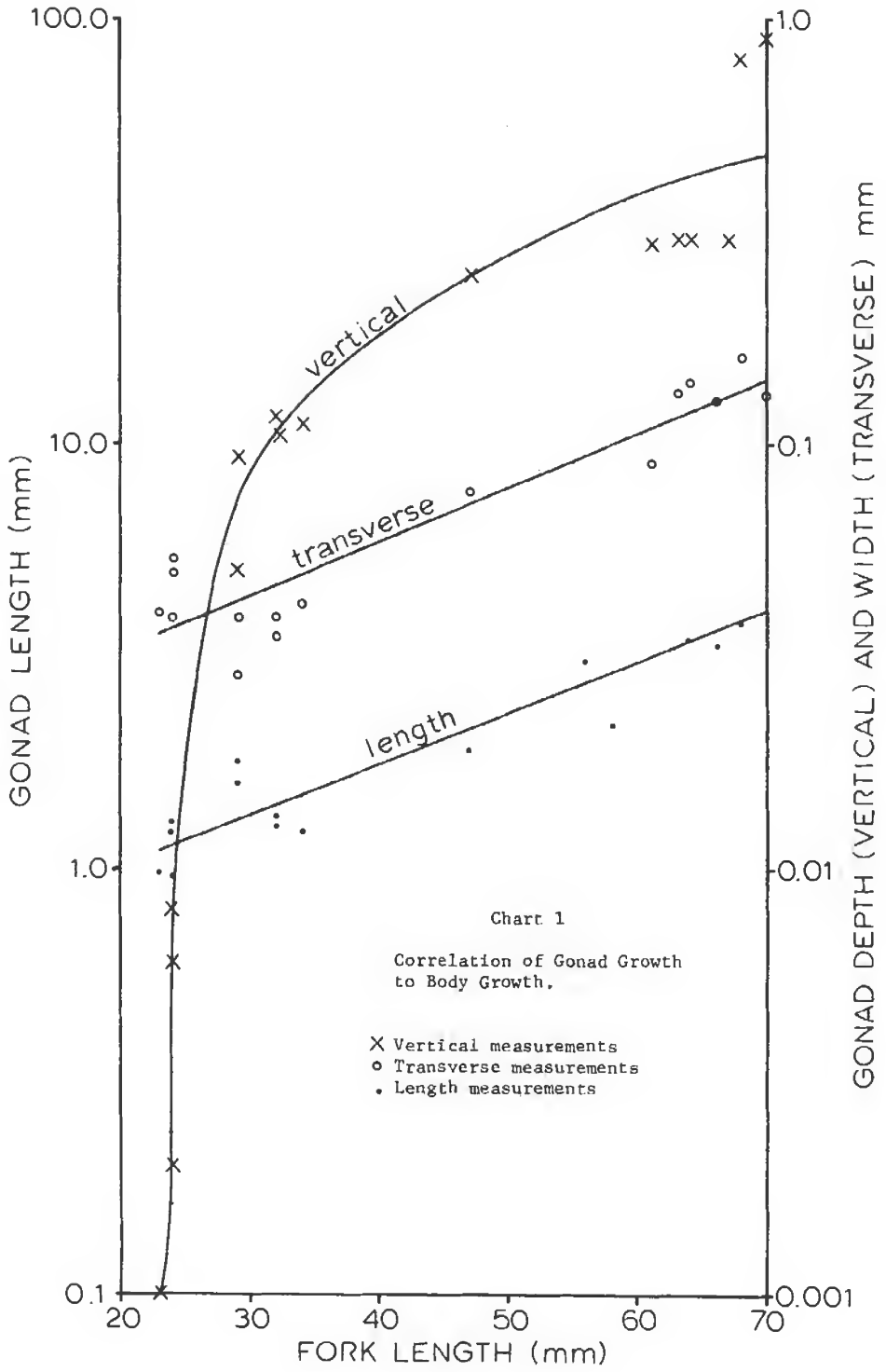


Fig. 21

PLATE 5


100.0
10.0
1.0
0.1
1.0
0.1
0.01
0.001
20
30
40
50
60
70
GONAD LENGTH (mm)
GONAD DEPTH (VERTICAL) AND WIDTH (TRANSVERSE) mm
FORK LENGTH (mm)
vertical
transverse
length
Chart 1
Correlation of Gonad Growth
to Body Growth.
X Vertical measurements
o Transverse measurements
. Length measurements

not observed to occur in the cytoplasmic field, it is postulated that, having attained this condition, they become more or less confluent with each other and are in this condition inseparable from the hyaloplasm. If such is the case, at least a quantity of the yolk present in the mature ovum is in an amorphous state, but it does not present the appearance described by Naumov (1956) in the herring. In the ripe oocyte of this fish he observed large, transparent lumps of the material which had sometimes fused together, a joining which he considered might have been due to fixation. Although this condition was not apparent in the menhaden, it may occur in some types of oocytes; for example, in the dab oocytes about which Wheeler (1924) reports that at maturity the yolk becomes more fluid and globules of the material coalesce to form larger drops. Fusion of the yolk globules at maturity has also been reported by Cunningham (1894) in the plaice, whereas in Zoarces the yolk is present as large spheres according to Mendoza (1939), who also describes a granular flocculent form in *Neotoca bilineata*. He also reports that in *Jenysia* and *Xiphophorus* the yolk is present as a single, large, solid mass. Craig-Bennett (1930) directs attention to the terms "primary" and "secondary" yolk as used by Hann, who recognized differences following the employment of Masson's Trichrome stain.

Insofar as could be determined, lipogenesis does not occur in Stage VI. At this period, the centripetal migration of the cortical fat globules which was in progress earlier has ceased. Since this inward movement was not total in its effect, the mature ovarian ova contain a relatively small number of sub-cortical large (8 to 12 microns), oval or spherical oil globules, some of which lie in conjunction with neighboring globules while others are more widely separated. They appear as colorless vacuoles in Plate V, Fig. 16. The intimate association of one or more smaller globules at the margins of some of the larger types suggests that a residual degree of coalescence is occurring. Internal to the sub-cortical layer of oil globules, a few globules are indiscriminately distributed in the yolk. Although variable in size, they are, with rare exceptions, smaller than those in the peripherally-located aggregate. In this deployment some occur adjacent to or in the evirons of the peri-nuclear globules which appeared during nuclear regression in Stage V. It is not thought that these residual globules occupying the intermediate zone between the cortex and the central oil mass will become permanently established at these points, but that they will slowly move centrally and ultimately associate themselves with the interior fat body. The vitelline areas of the ovum would then be entirely devoid of lipid elements.

The prominent internal lipid body (Plate V, Fig. 16) is typically located at the cell center in the area formerly occupied by the nucleus although it is occasionally eccentrically situated, a condition which may be caused by currents set up within the cell by the unequal rates of penetration of the fixing reagents. This is indicated by the fact that this condition was most common in menhaden that had been preserved intact or frozen prior to killing and fixing the ovaries. On the other hand, it is quite probable that its eccentricity is a transitory carry over of its displaced position at one side of the nucleus as commonly occurs in Stage V oocytes (Plate IV, Fig. 14). The oil body is comprised of many scores of individual oil globules which are

organized into a solid aggregate. Each of the elements generally retains its fundamental spherical shape, although this configuration is frequently modified by pressure to form polygonial types. Delicate membranes surround each entity, but the surface of the aggregate is devoid of any specialized investment. In the absence of such membrane, the spherical entities at the marginal interface collectively impart a scalloped configuration, and the entire body is suggestive of a morula. The individual moieties comprising the oil globule evidence little fluctuation in size, which may be interpreted as meaning that their subsequent coalescence or fusion will not occur.

VII. ATRESIA OF OOCYTES

Atresia or the absorption of teleost oocytes has repeatedly been described in the literature. Although its general occurrence is known, diverse opinions have been held regarding the maturity status of the ovary at the time it occurs, its functional significance, the factors which precipitate it, and the mechanics of the absorptive process. Stuhlmann (1887) has described its existence in immature follicles of *Zoarces*, which he designated as *"Zelldetritus"* or *"Galbertartigen Masse."* In the Pacific gunnard, *Leuresthes tenuis*, Clark (1925) having divided the sequential phases of oocyte development into immature, intermediate, and mature stages states that degeneration of unspent mature eggs occurs following the spawning season, while numbers of oocytes in the intermediate condition abort throughout the year. Wallace (1903) and Ghost and Kar (1952), working with *Zoarces* and *Heteropneustes fossilis* respectively, have reported its occurrence in all developmental stages, including quite immature oocytes.

Complete abortion of an entire crop of oocytes was not observed in *Brevoortia patronus*, and reference to the condition in other clupeids was not encountered in the literature, except for a statement by Naumov (1956) that in spent ovaries of the herring a reabsorption of unspawned cells occurred, a circumstance which, without further qualification, would include the incipient stages which in many fish, including the large scale menhaden, constitute a reserve for the following season. The extent to which catastrophic abortion occurs in other fish is not fully established, although it is apparently an uncommon phenomenon. It has been noted however in maturing oocytes and eggs in a spawning condition in *Zoarces* by Wallace (1903), Mendoza (1939) in the viviparous teleost *Neotoca bilineata*, and by James (1946) in *Lepomis macrochirus* and the large mouth bass, *Huro salmoides*. Wheeler (1924) was uncertain of the occurrence of total atresia in developing oocytes of the dab, although he observed the activity in numbers of residual ova remaining after spawning. Extensive, but not total mortality of unspawned mature ova has been observed by Cunningham (1894 and 1898) in ovaries of the plaice, although he considers it as an unnatural occurrence, and by Barfurth (1886) in unruptured follicles of the trout. Barfurth concludes that in such cases the degenerate masses may hinder the production of ova for the next spawning season and that the unripe condition may persist for two years or more.

Since most of the descriptions of atresia consider the frequency of its occurrence, the state of maturity of the cells involved, and the mechanics of reabsorption, little information has appeared regarding the specific factors which determine its onset. Since the activity has been shown to occur in cells during all developmental stages beyond the oogonia and first stage oocytes, and its extent to be partial or complete, it may be presumed that its precipitation may be effected either by generalized or localized factors respectively. In the former case, it must be presumed that a generalized "stimulus" is involved that has a broad range of influence on oocytes in every stage of development. This assumption would reduce the problem to two possibilities. Either the intraovarian vascular flow is diminished, which is not an uncommon condition in vertebrate organs, and would result in a physiological depression of the affected follicles and death of the contained oocytes and their subsequent reabsorption, or death is the result of an imbalance of the hormonal system. Of these alternatives an imbalance of hormones is indicated because of their established role in follicle, and therefore oocyte maintenance, and also because any reduction of the vascular flow sufficient to deprive the oocytes of materials essential to their existence would simultaneously hinder the removal of degenerative metabolic products. On the other hand selective atresia may be attributed to pressure atrophy which is prevalent in the ovary during the period that large numbers of oocytes are simultaneously undergoing substantial enlargement. This has been suggested as a contributing factor by Mendoza (1939), who observed that for the ovary to accomodate the number of eggs reaching maturity, there must occur a numerical decrease in some of the cells in the developmental stages. On the other hand, local pressure atresia cannot logically be implicated in those instances where large numbers of unspawned ova are reabsorbed subsequent to the termination of a spawning season. Because of the extensive mortality characteristic of these cases, it must be inferred that the precipitating mechanism originates in such a manner that its action is deployed to all susceptible oocytes. In the case of juvenile or adolescent fish that initiate the maturation process too late to be completed within the season, catastrophic atresia of a crop of immature oocytes results from the precocious onset of seasonal environmental conditions that are unfavorable to the continued activation of the pertinent endocrine organs to produce sufficient quantities of hormonal substances to force the maturation of the cells to the terminal state. This condition has been demonstrated repeatedly in many of the higher vertebrates.

The mechanics and physiology of reabsorption of atretic oocytes has received the attention of numerous investigators. In the intervening years since Barfurth *(op. cit.)* set forth his views on the events involved in their removal, the process has diversely been attributed to the activity of the follicle cells, cells of connective tissue origin, and to phagocytic cells derived from the vascular system. Prior to 1897 all of these criteria have been considered as playing a role of the removal of the aborted oocytes, which according to Cunningham (1898) includes the researches conducted by Brock, Emory, Owsiannikov, and others. The reabsorptive role of the follicle cells as the principal mediating factor has been emphasized by Cunningham *(op. cit.)*. In the plaice he found in the younger atretic oocytes an increased man-

ifestation in basophilia, the fusion of nuclei with the matrix of the cytoplasm. He attributed the removal of the detritus as being performed by these nuclei and identified them as arising from the follicular epithelium which had been derived from the adjacent connective tissue cells of the lamellar stroma. Further support of this interpretation has been provided by Wallace (1903), who observed that the follicular cells in *Zoarces* penetrate the zonal membrane and become phagocytes, sequentially instituting the reabsorptive process. He considered the role of the vascular leucocytes to be insignificant except possibly in the terminal stages. This interpretation agrees substantially with Ruge's concept (1889) that oocyte removal is due to an interaction of both leucocytes and follicular cells. On the other hand, considerable importance has been attributed to the leucocyte by Barfurth (1886) and Mendoza (1940). In *Neotoca* Mendoza (1940) observed that evidences of oocyte abortion is first evident within the atretic cell and that normally the follicle cells retain their peripheral position for some time while the cytoplasm of the egg breaks up. He concluded that it is likely the debris is removed in part by absorption, (the origin of the necessary enzymes not indicated), and in part by leucocytic phagocytosis. Since there continues to exist a diversity of opinion as to the cellular and bio-chemical aspects involved in these regressive activities, Wallace's comments in 1903 on this uncertainty remains to a large extent still pertinent when he stated that " . . . the earlier attempts to explain this occurrence . . . were very wide of the mark." In *Brevoortia patronus* abortion of oocytes in Stages I and II was not observed. It is held that because of their immaturity they exist as stable, undifferentiated cells that have not responded to the oogenetic control mechanisms involved in he maturation process but which at the same time culminate in the establishment of the unbalanced conditions associated with specialization of the oocyte. Under these conditions Stage I and II oocytes exist indefinitely as primitive cells.

Because of the cytological diversity of the oocytes in Stages III through VI and the differences in the degree of follicular development during the period, considerable variations are manifest in the images presented by the aborted cells and the events which transpire during their dissolution. These factors require that separate consideration be given to the atretic activities as they appear in Stage III oocytes in contrast with the cells in later stages after the vitelline materials have made their appearance.

(1) Stage III Atresia

Catastrophic atresia of Stage III oocytes rarely occurs in the mature, large-scale menhaden. The author has observed substantial atresia of these cells in a few juvenile ovaries which had initiated a slight degree of maturation activities during the late summer and autumn but were not able to stabilize or maintain the cells to the onset of the ensuing spawning season. In these ovaries the oocytes appeared to be in an early phase of Stage III, and the abortive activities assumed a dominance in December and January. Most juvenile ovaries, however, pass through this phase without the involvement of any significant degenerative changes, although an occasional aborted cell

may appear in the ovary during any month of the year. In contrast to the season and the magnitude of cells aborted in juvenile fish as described above, females which have spawned abort Stage III ova most frequently in the interval between approximately April and September, during which time the ovary is in a relative quiescent state. The minimal atretic activity of oocytes in functional ovaries occurs during the spawning period and for a short period thereafter.

Absence of yolk, the presence of few or no lipid masses, and a follicle devoid of highly organized epithelium in this stage provides a regressive picture unlike later stages. The initial indication of the onset of atresia involves a rapid change of the cytoplasm of the early phase oocyte from its usual hyaline basophilia to a flocculated, dusty blue or greyish lavender aspect as illustrated in Plates V and VI, Figs. 21 and 22 (after Heidenhain). In cells which are more advanced and which are normally characterized by a pattern of intensely staining material embedded within the less basic cytoplasm (Plate III, Fig. 12), the contained substance loses its pronounced affinity for these stains. Sequential to a considerable depletion of the basophilic condition, the cytosome is comprised in all aborted Stage III cells of a myriad number of flocculated, grey-blue granules which lie in a colorless ground matrix, and are remarkably of a uniform size (0.1 to 0.2 microns in diameter). Although they tend to be homogeneously distributed, frequent clumping occurs which gives rise to small masses of less than 1 micron. Oil globules, if present, disappear abruptly by a process of progressive diminution, as though actuated by inter-cellular enzymes.

A series of nuclear changes are initiated concurrently with or soon after the onset of the cytoplasmic disorganization. These are introduced by the disappearance of all chromatin; a profound enlargement of the nucleolus (if present); the development of a state of confluency of the proto-vitellonucleoli; and a transition of the original nuclear sap into a somewhat acidophilic substance. Very early the proto-vitellonucleoli lose their form and their substance runs together to create a continuous slightly hypertrophied ring on the inner face of the nucleus. The structure is strongly basophilic and has an initial wall thickness of from 3 to 5 microns. Occasionally it presents an enlarged trilaminate shell, of which the outer layer becomes rough-surfaced and either presents a basophilia or occasionally an acidophilia. In the latter form the middle layer is somewhat chromophobic and slightly thicker than the adjacent layers, while the innermost stratum assumes basic properties to a variable degree. The internal margin of the deeper layer is either uniform or slightly undulating, but ragged or serrate on its opposite face which is in juxtaposition with the mid layer. Regardless of the form it assumes, the modified nuclear wall remains intact as regards size and position for only a brief period as indicated by the small numbers of cells having nuclei in this condition. By rapid hypertrophication it expands both as to its diameter and the thickness of its wall into the state illustrated in Plate V, Fig. 21. The subsequent disintegration of the ring does not occur uniformly as indicated by the presence of places where its substance is interrupted and incomplete. In such cases, it develops in its internum a few small achromatic areas, which increase both in number and size until the body completely disappears. The initial events

are shown in Plate V, Fig. 21. Plate VI, Fig. 22, shows the oocyte after the absorption of this body is complete.

The nucleolus, if present, enlarges materially so as to approach the size of the original nucleus. For a period it retains a basophilia. Very soon, however, an achromatic area appears in its center which spreads to the periphery resulting in the disappearance of the body. Since at this time external cellular elements have not penetrated the aborting oocyte, the lysis of the nuclear bodies is effected entirely by enzymes, contained with the oocyte. The events terminating in the precocious removal of the nucleus now presents a state in which the cytoplasmic matrix is enclosed in a quite undeveloped follicle.

After the completion of nuclear assimilation, the absorption of the cytosome progresses in a more leisurely manner. Its removal is introduced by a peripheral invasion of a small number of slightly modified fibroblast-like cells. They are best recognized immediately after their appearance near the periphery of the oocyte since their shape and internal structure ultimately becomes so highly modified that their ancestry cannot be definitely identified. In their earliest form they have a mean diameter of about 5 microns. The centrally-located, spherical nuclei, measuring about 2.5 microns, contain a single, small chromatin granule at the mid-point, from which a few delicate fibers radiate through a colorless nuclear sap to the distinct, but delicate karyotheca. The achromatic cytoplasm is distinguishable from the nuclear sap only by the presence of the nuclear wall. In favorable preparations, from two to six of these cells occur in the periphery of the oocyte cytoplasm immediately under the somewhat flattened follicle membrane, which, however, appears to remain intact.

A number of possible alternatives may be advanced to account for the origin of the invading elements. Thus it may be theorized that they are derived either from the vascular system, the primitive follicle cells, or the stromal fibroblasts. That they are a type of vascular leucocyte would presuppose their migration through the investing follicle. Although this may undetectibly occur, the cytology of the elements in the regressing cells is quite unlike the ichthyoid forms of leucocyte. Secondly, their origin may be attributed to a detachment of some of the follicle cells and their migration into the mass. This would produce interruption of the follicle membrane at the points of detachment although it is conceivable that a shifting of cells adjacent to their points of departure would reconstruct a continuous investment. This possibility is tentatively supported by the occasional occurrence in the follicle of a few hypertrophied cells which may be considered as the progenitors of the elements invading the oocyte, although it is contra-indicated by the persistence of an intact follicle throughout the period of absorption (Plate VI, Figs. 21 and 22). Finally some evidence suggests that these entities may be derived from fibroblasts of the ovigerous lamella. Thus, there occurs on the outer face of the follicle a few cells which are transitional between the typical fibroblasts and the phagocytic cells in the aborted oocyte. Involvement of these cells in the absorptive processes would pre-assume their penetration of the follicle membrane. However, considering the comparative cytology of the contingent stromal cells and those found at the surface of the aborted oocyte, it is the author's opinion

that the phagocytic cells originate from fibroblasts. Although this represents a view which was not encountered in the literature, it appears to possess validity in view of the stability of the follicular components, as previously stated, the justification given for excluding vascular leucocytes, and the known potentialities of the fibroblastic cell types to redifferentiate. The mode of entrance of these cells into the atretic material was not established with certainty. Although in the terminal state of absorption the follicular membrane exhibits some discontinuity,the phagocytic cells in the oocyte usually occur in areas where the membrane appears intact. It is to be pointed out, however, that at Stage III the follicular investment is very primitive so that it is not completely organized on all surfaces of the oocyte. Under these curcumstances the entrance of the lytic cells occurred at openings which were not visible in the sections studies. Reduplication of the phagocytic cells after entrance into the oocyte was not observed.

Reabsorption of the atretic mass occurs only at its periphery and is furthermore limited to the environs of the phagocytic cells. In such regions the cells lie in conforming lacunae which intrude into the surface of the granular oocyte substance. This pattern of encroachment is repeated at several points around the oocyte margin. With the establishment of this pattern, the achromatic cytoplasm of the invading cells almost immediately begins to display an intense basophilia. This transformation may be limited to one or more particles measuring from 1 to 2 microns in size, or the change may occur as a generalized state throughout the cytosome. The enclosed substance is amorphic and with iron hematoxylin it stains a deep blue-black so that its intensity is many times greater than the adjacent grey-blue cytoplasmic remnants of the oocyte (Plate VI, Fig. 21). This suggests the intra-cellular elaboration of a product the source of which may be the oocyte ribosomes, or the associated intermediate or degraded RNA or RNP materials. Regardless of its origin, it soon occupies the entire interior of the phagocytic cells and effectively occludes their nuclear and other internal structure. In a few instances, a spherical or oval, light blue internum is visible, that may be construed as being the phagocyte nucleus. As this activity continues, it is accompanied by a transformation of the originally spheroid cell to oval, rod-shaped, or irregular patterns. The visible state of these bodies is questionable in that they may have been destroyed as a result of their activity.

Although proliferation of these cells was not observed, the increase in numbers of deeply staining peripheral entities of the approximate size, form and position with respect to the earlier units suggests its possible occurrence, although it is also recognized that the increment may also be due to a supplemental addition of elements from the stromal connective tissue cells which is obscured by the presence of the existing dark (cellular?) masses. As peripheral absorption proceeds, the basophilic masses become more extensive over the diminishing oocyte surface and assume a more or less confluence with each other. Since the rate of penetration of these masses into the internum is not synchronized, deep metastases develop which divide and subdivide the remaining atretic mass into smaller and smaller areas until it is completely obliterated.

Near the termination of the phagocytic activity the connective tissue follicular investment becomes thinner and disorganized. Its cells round up somewhat and become incorporated in the ovigerous stroma, where they are lost among the fibroblasts. The ultimate fate of the phagocytic cells burdened with the deeply basophilic material derived from the disorganized cytosome of the oocyte was not determined. During the mid or late phases of regression, many of the phagocytic elements develop a strong eosinophilia. Although a decrease in the staining intensity of both basophilic and eosinophilic types and their subsequent migration into the adjacent stromal complex was noted their further history could not be determined.

(2) Atresia — Yolk Stages

The form of atresia for which an account is given in the following paragraphs occurs in eggs of Stages IV, V, and VI. The regressive activities not only involve the cytosome and the nucleus, as was the case in Stage III oocytes, but must include a consideration of the fate of the vitelline and oil bodies.

Oocytes in these stages abort from late summer until after the termination of spawning. The process is rarely observed in eggs in which yolk formation was just beginning (Stage IV), since, as mentioned above, the interval during which the egg remains in this condition is quite abbreviated so that the oocytes quickly pass to the following stage. It is difficult to distinguish Stage V and Stage VI eggs after absorption has progressed somewhat, for which reason its relative occurrence in each of these stages cannot be stated with certainty. The study presented some indication, however, that it occurred more frequently in Stage V oocytes prior to spawning, but that during and after ovulation cells in Stage VI became more involved. The period required for the complete removal of these aborted oocytes is of greater duration than in yolkless cells. In ovaries which were known to have ovulated during the winter, remnants of aborted follicles can be found as late as May or June. It seems that after the process has been precipitated the initial phases proceed with great rapidity, but that the rate progressively diminishes.

The onset of atresia is marked by a precipitous modification of the *zona radiata* and by a hypertrophy and cytological alteration of the cells constituting the follicular coat of the follicle. During the earlier phase of this event, the follicle cells remain in a single layer, but after a short interval they become transformed into a wall from two to three cells in depth. With this occurrence the thickness of the investment may become as much as 10 to 15 microns, or comparatively two or three times the normal membrane. A marked cytological similarity exists between the cells in the various layers. Compared to normal follicle cells the nuclear changes are marked by some dilation, an almost complete disappearance of their contents so that they appear empty with only one or two small, occasional basophilic inclusions which may be vitellonucleoli, and an increase in the distinctness of the karyotheca. Coincidentally with these transformations the cell enlarges from about 5 to 9 microns. This increase is not the product of the accumulation of more cytoplasm but of a form of vacuolation. Whereas the cytosome in its original state

was homogeneously finely granular, the effect of impending atresia is the establishment of a reticulated or shredded matrix which encompasses many variously sized and shaped vacuolate components. As a result of the development of the cytoplasmic strands and in the absence of a visible, limited cell membrane, the layer or layers soon assume the form of a continuous, uninterrupted mass of shredded material containing occasional vague nuclei.

While the events described above are progressing, retrogressive activity is evidenced by the *zona radiata externa* and the *zona radiata vera*. The latter component exhibits a marked swelling which may double its thickness. Because it is erratically initiated throughout the extent of this sheath, disassociated areas of its inner face assume the shape of a few or many inwardly-directed mounds or nipple-like elevations which may be contiguous with each other or irregularly separated. Such elevations force the underlying vitelline membrane into the substance of the cortical cytoplasm of the oocyte. At first, the interfaces between these layers is sharply defined, but eventually they become vaguely confluent. The hypertrophication of the radiate membrane not only displays an increase in its width but is also reflected by a marked circumferential increase in size. The circumferential increment causes the entire membrane to be thrown into undulating folds as illustrated in the large central cell in Plate VI, Fig. 24, the inward extremities of which push somewhat into the cortical substance of the oocyte. Occurring concurrently or immediately following the events just described the oocytes display many minor differences in their further regression. In nucleated oocytes (Stage V), the body precipitously undergoes disruption and it contents are quickly disseminated in the cytosome and become no longer evident. Because of the early disappearance of the nucleus, oocytes of Stage V are not always distinguishable from those of Stage VI, and after the disappearance of the central oil globule that characterizes the latter stage their separate identities are not distinguishable.

While the zonal membranes are in the condition described above, the *zona radiata vera* for a short interval retains its normal acidophilic response to stains. Soon, however, disassociated areas of basophilia begin to appear within its internum. The size and morphology of the basophilic zones are extremely variable. In general, they appear in sectioned material as arcs which occupy about the mid third of the width of the *zona radiata vera* and are surrounded internally and externally by a thin layer of unaltered eosinophilic material. By the time the *zona radiata vera* has initiated the development of the internal basophilia, or in other instances where the process is incomplete, the membrane appears in sectioned material to break apart. The mechanics of membrane disjunction is thought to involve physical rather than chemical forces. This is suggested by the appearance in areas which have not yet shown a shift to the basophilic state of a considerable number of oval or spindle-like cavities of one to three microns in length. The long axis of these vesicles conforms to the circumferential direction of the membrane. Since their occurrence coincides with the hypertrophy and in some cases the folding membrane, it is probable they are derived from the system of radial canals which, due to the effect of distorting forces acting on the membrane at this time is causing the micro-tubules to be stretched along planes

coinciding with the zonal layer. Their presence is transitory. Because they disappear during the interval when the *zona radiata* fragments, they are thought to play an important role in disruption of the membrane by establishing points of fragility.

The basophilic internum in the various segments continues to enlarge so that it progressively replaces all of the original eosinophilic material of the *zona radiata vera*. The two components of the zonal membrane then become indistinguishable since they are still in conjunction with each other and evidence the same degree of basophilia. As they deploy toward the internum of the aborted oocyte, they irregularly fold upon themselves in such a manner that the entities assume the appearance of irregular basophilic masses which are disposed promiscuously around the atretic cytosome (dark bodies at center right in Plate VI, Fig. 23, also Fig. 25). These sink into the deeper layers of the cortical cytoplasm and commingle with the vitelline substance. During their inward migration they pass through the region occupied by the vitelline membrane. The fate of this membrane is uncertain, although its distinct features disappear rather abruptly and it becomes structurally and tinctorially identical with the cortical cytoplasm.

The breaking apart of the *zona radiata* is of utmost significance in the further absorption of the oocyte. At the interstices created by its disassociation, there will soon occur massive invasions of the cells of the follicular membrane. Within a short time they become distributed either in groups or singly among the more peripheral yolk globules or along the faces of the zonal fragments. This situation is illustrated in Plate VI, Fig. 26, which includes only a small part of the oocyte. The follicular epithelium is seen as a dark band of cells in the lower part of the photograph just above the light space that separates the aborted cell from a Stage III oocyte at the bottom. Because the atretic cell contour was somewhat "U" shaped in the area photographed due to compression by other near-by oocytes, the follicular surface of the same cell is visible again at the upper left hand corner. Within the expanse of the cytoplasmic matrix, two irregular shaped homogeneous gray bodies can be seen. These are fragmnts of the *zona radiata*. Lying throughout the cytoplasm are two or three score dark circular nuclei of the follicular cells that have migrated to the internum. These cells are dispersed so that some of them lie adjacent to the zonal fragments and the yolk bodies. (The yolk granules are represented as moderately large light grey bodies, cf. *infra)*. A few of the invading cells are shown to be contiguous to or within the zonal masses. The figure also shows the continuity that occurs between the cells of the follicle layer and the free cells in the interior of the oocyte.

With the completion of the disorganization of the *zona radiata* and the intrusion of the follicular cells, the aborted oocyte presents a complex comprised of a mass of indistinct cells of follicular origin, irregular bodies derived from the *zona radiata,* a moderate quantity of granular, acidophilic cytoplasm, and a mixture of vitelline bodies. The oil globules precipitously disappear in the same manner as in aborted Stage III oocytes, and without the apparent intervention of phagocytic activity. The vitelline bodies show evidence of a marginal indistinctiveness and a confluence with each other. They stain var-

iously weakly acidophilic or basophilic. Often their internum, which may be entire or broken into two or four fragments, is for a time basophilic, while the cortical zone responds to the acid dyes. In the process of removal of these bodies, their final absorption can occur only when their substance is in an acidophilic condition.

Selective absorption of the bodies within the oocyte was not observed. Rather there appears to be a simultaneous removal of yolk bodies together with the submerged portions of the *zona radiata.* In late stages of oocyte removal, parts of both of these materials may be present, although in some instances the zonal fragments are absent. The exact mechanism of the removal of the debris could not be established. Since vascular buds do not enter the remnants of the follicular investment until after the appearance of large numbers of scavenger cells in the detritus, the hemal system does not appear to be implicated as a source of phagocytes during most of the period of oocyte removal. Phagocytosis by the cells derived from the follicle was not suggested on the basis of an absence of atretic material within their cytosomes, although the possibility exists they are involved, but that the process is obscured by chemical or physical alteration of the debris during ingestion. Rather it appears that enzymes derived from the follicle cells act extra-cellularly on the yolk, cytoplasm, and fragments of the *zona radiata.* The validity of this assumption rests upon 1) the juxtaposition of the follicle cells to the yolk bodies or the masses originating from the *zona radiata;* 2) that at these points the substance of the detritus is always in a comparable acidophilic condition; 3) that with respect to the particles of zonal origin the follicle cells frequently occur in bays or lacunae conforming to the shape of the cell; 4) the cytosome of the follicle cell retains its vesicular morphology throughout the process; and 5) the not infrequent occurrence in the detritus of yellow-brown pigmented objects having irregular shapes and being slightly larger than the contained cells. These are recognized as metabolic by-products of extra-cellular lysis.

Sooner or later the above described activities progress to a stage in which the volume of the aborted oocyte is greatly reduced. A few dozen yolk granules occur in a section instead of hundreds. When this phase is attained, numerous capillary buds or aggregates derived from the lamellar stroma push into the connective tissue investments which originally were present on the outer surface of the cellular layer of the follicle. Some shrinkage now occurs in the diameter of the connective tissue tunic, but since the change is not at rapid as the rate of diminution of the oocyte debris, a considerable space exists between them. The blood cells are at first confined to these new channels. In the final phases of absorption a few leucocytes are found in the space between the peripheral capillaries and the residual debris, and in rare instances an occasional hemal element is encountered in the detritus. It is doubtful from their cytological appearance and distribution that their role is one of actual phagocytosis, although they may be engaged in some form of extra-cellular digestion of the oocyte substance.

Shortly after the leucocytic invasion, there gradually develops in the area occupied by the residual complex comprised of the remaining follicle cells, the oocyte debris, and the hematic elements

an irregular, reticular network of fine connective tissue fibers. These elements are not numerous and are disposed throughout the detritus without any specific organization. Associated with the fibers is a cell type that was not previously present. These occur in varying numbers. They are oval or elongated with a distinct eosinophilic nucleus and a finely granular cytosome possessing a less intense but similar staining matrix. The approximate cell size is four microns. In all respects they closely resemble the fibroblasts present in other areas of the ovigerous lamellae. Because the outer investment of connective tissue remains at some distance from the residual atretic complex, and therefore is not spatially in a position to contribute these cells, it is believed that their origin is either from the leucocytes or the follicle cells which initially entered the substance of the aborted oocyte. While transitional forms were not specifically identified, their temporal appearance closely follows the leucocytic invasion, thus providing indirect evidence that the progenitor is the white blood cell. This, however, leaves unexplained the fate of the follicle cell whose numbers gradually diminish as the remnants of the oocyte are absorbed. This uncertainty is furthermore complicated by the appearance in the mass during the final stages of a number of cells intermediate in morphology between the earlier hypertrophied follicle cell and the fibroblast. If it is assumed that the fibroblasts present in the terminal, internal connective tissue mass are all derived from the numerous follicle cells which were originally associated with the absorptive process, it becomes necessary to account for the substantial reduction in their numbers, since at the time of complete absorption of the oocyte the total of cells present in this confined area is materially less than those originally contributed by the follicle. Because cells of follicular ancestry were never observed to leave the aborted mass, an activity which might otherwise explain their diminution, it appears that the most valid explanation of the roles played by the cells contributed by the follicle and the leucocytes is that the follicle cell is entirely responsible for all activities involved in the digestion of the *zona radiata* and other constituents of the aborted cell, but that the leucocyte possesses the dual potentiality of destroying the degenerating follicle cells, after which a part of the leucocytes differentiate into fibroblasts while others return to the vascular system.

In summary, it has been shown that in the menhaden ovary the process of absorption of atretic oocytes is basically identical regardless of the age of the oocyte. In this respect, the first evidence of regression is always manifested by nuclear changes that soon result in the liberation of its substance into the cytosome. The chemical disintegration of the cytoplasm and its enclosures in all aborted cells is accomplished by extracellular enzymes rather than by phagocytosis. The cells implicated in the lytic removal of the oocyte remnants are always of fibroblast origin, i. e., they are either invading fibroblasts as in the younger stages or modified fibroblasts serving as follicle cells in the case of older oocytes. The role performed by leucocytes is confined to the terminal re-establishment of stromal tissues.

For an interval subsequent to the complete removal of the substance of the aborted oocyte there occurs in the area it formerly occupied an island of newly formed connective tissue which remains

separated from the stromal matrix of the lamella by a contiguous, empty zone which is in turn surrounded by a loosely organized layer of fibrous connective tissue as described above. The cavity separating the internal fibroblastic mass from the externum is progressively obliterated by the encroachment of the blood vessels and the outer connective tissue tunic and also in part by a supplementation of the elements of the central connective tissue body. Ultimately the external and internal tissue become applied to each other in such a manner that the fibers of one become continuous with those of the other. At this stage the follicle has been completely absorbed and the elements present are to be considered as comprising a portion of the connective tissue stroma of the ovigerous lamella. Nevertheless, it is possible to some degree to identify for prolonged periods the connective tissue elements which arose in the residual oocyte detritus from the type derived by stromal invasion because of the greater density of the former. Following an unknown lapse of time, the distinction becomes progressively more vague until the dual origin of the elements is obliterated and the area assumes the aspect of the general stroma.

VIII. INVESTMENTS OF THE OOCYTES

A number of investments are associated with oogenesis of fish oocytes. These show some variability in number and structure in the different species of fish but possess two characteristics which are universally applicable. In the first instance, the greater number of the membranes present are functionally concerned with the transmission of synthesized and unsynthesized substances essential to the growth and differentiation of the oocytes. Additionally, one of the investments later becomes involved in the formation of the fertilization membrane.

The variable terminologies and classifications which have been assigned to these structures by Mark, Eigenmann, Emory, Schraff, Heape, Caldwell, and Fulton have been reviewed by Wallace (1903). In the case of the Gulf menhaden, the following classification appears to be the most descriptive of the types and relationships of the oocyte investments:

A. Primary membranes. (Those which are retained in whole or in part by the ovum after ovulation.)
   1. Plasma membrane. (An interface cytoplasmic membrane lying external to the vitelline membrane.)
   2. Vitelline membrane. (Occurring at the oocyte surface.)

B. Secondary membranes. (Those which are retained within the ovary as remnants of the follicle after ovulation, and identified in sequence from the exterior to the interior.)
   1. Follicular connective tissue fiber tunic. (A loosely organized association of connective tissue fibers.)
   2. Epithelial follicular membrane. (The principal investment as regards synthesis and transport of materials essential to oogensis.)
   3. *Zona radiata externa.* (An outer component of the *zona radiata.* Basophilic.)

4. *Zona radiata vera.* (The inner component of the *zona radiata.* Acidophilic.)

Considering the temporal development of these investments, it can be stated that the primary structures make their appearance prior to the secondary membranes. This condition may not be particularly obvious in the youngest oocytes because of the feeble tinctorial responses of the elements and because the components are so delicate they are difficult to identify. In the following sections, follicle development will be correlated with the maturation stages of the oocytes.

Stage I: A true vitelline membrane does not occur at the surface of these oocytes. There is present, however, a poorly defined, peripheral condensation which may or may not be discernible in different preparations. When present, it varies in thickness from the limits of visibility to 0.2 microns in thickness. It is most effectively demonstrated by Cajal's trichrome method, following which its substance seems to be of a finely granular nature. Because of its immaturity, it cannot be specifically recognized as either cortical cytoplasm or vitelline membrane material. It is logical to assume, in view of the primitive state of the cell, that the vitelline membrane is incapable of precociously differentiating and that the substance is therefore cortical cytoplasm.

Lying on the margins of the oocyte, the stromal connective tissue becomes loosely associated with the germ cell (Plate II, Fig. 9) to establish the primordium of the follicle. Although no distinct demarcation exists between the general connective tissue of the lamellar stroma and the elements which lie in contact with the oocyte, slight cytological differences can be observed. Despite the delicacy of the fibers in both areas, they tend to attain predominance near the oocyte where they are roughly oriented in the form of a concentric, undulating pattern wherein the fibers frequently intertwine with each other. Since the circumferential fibrous zone gradually integrates with the randomly disposed fibers of the stroma, its thickness can be stated only in a general way to be between 0.3 and 0.5 microns.

Cellular differences occur between the fibroblasts of the stroma and those lying in the environs of the oocyte, but as in the case of the related connective tissue fibers, the transition is gradual. Typical stroma fibroblasts are multi-shaped, have rather imperceptible cell boundaries, and are usually from 3.0 to 5.0 microns in diameter. Enclosed in the weakly stained cytosomes are well-defined, uniform sized nuclei containing unstained or dimly stained karyoplasm embedded in which are six to twelve or more small and irregular chromatin particles. In contrast, the connective tissue cells as they approach the periphery of a Stage I oocyte, begin to elongate, at first slightly and subsequently more so as they come to lie in closer contact with the germ cell. Their long axis becomes noticeably arched as they approach the oocyte. In conformance with the shape of these cells, the prominent nuclei are elongated and crescent-like. The internal aspects of these karyosomes are similar to those of the outlying fibroblasts.

In summary, Stage I is passed over without formation of any of the membranes of the oocyte. The principal activities in this direc-

tion are confined to the provisional organization of the connective tissue entities which are destined to form the follicle.

Stage II: Since this stage is transitory and does not involve extensive cytoplasmic maturation, very little progression occurs in the elaboration of the oocyte investments. The encapsulating connective tissue fibers become somewhat more abundant (Plate III, Fig. 9, darker cell), although their fundamental arrangement remains unchanged. While the thickness of the layer may increase to about one and one-half of its original extent, there is no suggestion of its sequential differentiation into the characteristic follicular investments. The cytosome of the oocyte also remains passive as regards the formation of a vitelline membrane.

Stage III: Actual follicular differentiation begins during the early part of this stage and is correlated with the initiation of cytosomal differentiation, i. e. the assumption of a generalized basophilia and lipogenesis. Before the oocyte enters the subsequent stage, the young follicle has become a two-layered structure consisting of the incipient epithelial follicular membrane and the primordium of the *zona radiata*. Follicular epithelium is not present on the surface of the oocyte during the earlier part of Stage III. The period of its organization begins at about the time the germinal component has attained an intense basophilia. The first indication of its development is the appearance on the outer surface of the oocyte of an occasional cell that is closely adherent to the surface. These cells are cytologically similar to the fibroblasts that occur in moderate numbers in the environs of the oocyte and from which they were derived. Almost immediately the presumptive follicle cell becomes greatly flattened against the oocyte surface although it is not unusual to see its margin reflected from the oocyte surface in the manner of a tangent. This imperfect attachment is only a temporary condition that occurs while it is still in the process of conforming to the curvature of the oocyte. During this interim there are considerable areas of the germ cell that are devoid of the external investment. Soon, however, other cells appear at the surface of the oocyte so that it becomes completely ensheathed. It appears from the material that the supplemental cells are derived from the stock of fibroblasts rather than by replication of the original adherent cells. With the completion of these events, a primitive follicle has been created.

At the time the components of the follicle have reached the stage of organization described above, they appear as a continuous entity in which cell boundaries cannot be found. The thickness of their cytosomes is hardly within the limits of visibility. Their basophilic nuclei are compact and although flattened against the oocyte rise slightly above the cytosomes. Because of this the outer surface of the follicle appears slightly undulate.

Very little further development of the investment occurs during Stage III. The cells become slightly less flattened and the nuclei lose their basophilia so that their internal structure becomes visible. Within the distinct nuclear membrane there occurs a vesicular arrangement of the components and numerous small chromatin masses. The achromatic cytosomes are finely granular. Plate IV, Fig. 17,

shows a general view of the follicle on the oocyte in the upper part of the illustration. The oocyte represented is about midway through Stage III.

Underlying all areas of the epithelial investment a very delicate non-cellular layer is present which represents the appearance of the *zona radiata*. It is amorphous and reacts weakly to acid dyes. At the termination of Stage III, it attains a uniform thickness of slightly more than 2 microns. In some preparations suggestive evidence is presented that the structure is composed of circumferentially arranged, masked parallel bands of broad fibers or layers of a material physically resembling regular dense collagenic connective tissue. In areas where the techniques caused its separation from the margin of the oocyte, it appears to possess on its inner face a thin layer of denser material. This may be the result of the separation of the delicate vitelline membrane from the oocyte and its adherence to or fusion with the zonal anlage. In other areas where this has not occurred, the vitelline membrane is barely discernible as it lies on the surface of the cortical cytoplasm. At this period this membrane does not yet evidence a recognizable difference in staining properties from the cortical substance, except for a minimal increase in chromophilia.

Stage IV: Throughout this abbreviated stage the oocyte investments undergo progressive, minor differentiations of the layers developed in the preceding stage. Correlated with the institution of the process of the provisional yolk elaboration, the single layered epithelial follicular membrane increases in thickness to a maximum of about 6 microns, which represents a two to threefold increment (Plate IV, Fig. 17, lower and upper left oocytes.) With this development, the cytology of the cytosomes is unaltered, and their intercellular membranes are only suggested. The internal faces of the follicle cells which rest upon the incompletely differentiated *zona radiata* show some irregularity. The limiting membranes at this surface are comparatively more distinct than those separating one cell from another. The superficial cellular margin is represented as a thin layer of cortical cytoplasm rather than a well defined membrane. The interiors of the cytosomes are somewhat finely vacuolated and present a "washed-out" appearance.

Progressive nuclear enlargement in the follicular cells occurs so that they attain a diameter of about 6 microns, meanwhile undergoing a change from the earlier flattened discs to ovate discs. They generally lie at the cell center. Cytological changes from the earlier forms are insignificant.

The developing *zona radiata* at this period shows tinctorial evidence of differentiating into two inseparable stratifications, the *zona radiata externa* and the *zona radiata vera*. The entire membrane increases in thickness from about 2 microns to 3 or more microns. The *zona radiata externa* now develops a basophilia which completely encircles and encloses an inner ring of the acidophilic material constituting the *zona radiata vera*. Individual follicles evidence considerable variation in the respective thickness of each of the zonal components. The extreme condition, which is encountered rarely, involves complete replacement of the acidophilic part by the basophilic counter-

part. Under these circumstances the condition seems to be temporary and reversion of the tinctorial pattern to the original condition occurs during subsequent stages. Both components are amorphic throughout Stage IV and fail to display any suggestions of the internal structure.

The presence of the plasma membrane is not yet indicated. Although the vitelline membrane is still vaguely foreshadowed by its greater density, it does not occur as a distinct structure.

Stage V: Correlated with the accelerated rate of deutogenesis and lipogenesis characteristic of this stage, the follicular epithelial and zonal layers show a marked development.

The unstratified layer of follicular epithelium increases in thickness as the stage progresses and at its termination varies between 8 to 10 microns. Its cellular elements assume a cuboidal aspect as illustrated in Plate V, Fig. 18. The large oval or spherical nuclei are slightly apical in position, seeming to lie almost in contact with the cell membrane. Occasional small masses of chromatin lie against the prominent nuclear walls, otherwise they are structureless. The cytosomes appear foamy and vacuolated the elements of which progressively increase in size toward the basal regions of the cells adjacent to the *zona radiata*. Because this margin of the cells is adherent to the *zona,* and due to the apparent absence of basal membranes, considerable numbers of the vacuolations appear to be in contact with the zonal substance.

Zonal membrane development occurs so rapidly at the onset of this stage that the structure becomes fully functional in the earliest phase. Three views at different periods during the interim of the fifth stage are shown in Plate IV, Fig. 14, and Plate V, Fig. 18. The superficial *zona radiata externa* is extremely basophilic and of a constant thickness of about 1.6 microns. It is structureless internally except for an abundance of radial striations which pass through the matrix and which have been shown by E/M studies to be canals. These canals are less than 0.2 microns in diameter and in sectioned material are seen to be spaced uniformly at intervals of 0.6 microns. Their external apertures are intimately associated with the *zonal* faces of the cells of the follicular epithelium. Sections which happen to pass tangentially through the zonal layer so as to cut through the canals in a cross-sectional aspect reveal a regular geometrical arrangement of a polygonial nature. The *zona radiata externa* is bound firmly to the *zona radiata vera*. It consists of an amorphic hyaline acidophilic matrix, uniformly about 5.5 microns thick, and is permeated by indistinct radiations representing a continuation of the canal system of the *zona radiata vera*.

Internal to the *zona radiata vera* there begins to appear during the later interim of this stage a circumferential zone whose width is essentially 1 micron or slightly more. Its appearance in fixed material is such as to suggest the inclusion of a viscous, semi-fluid *in vivo*. Not infrequently a small number of minute granules are oriented in a radial pattern that suggests the existnce of diffusion currents between the canals of the *zona radiata vera* and the vitelline membrane of the oocyte. This morphological entity is the plasma membrane, which

because of its apparent high degree of viscosity is retained in whole or in part by the ovum at ovulation.

The vitelline membrane is definitely established during this stage, although its terminal differentiation is not accomplished until later. As shown in Plate IV, Fig. 18, the demarcation is rather sharp between this membrane (black line) and both the adjacent outer plasma membrane and the inner cortical cytoplasm. Whereas in the previous stage its lack of definition precluded exact measurements, it now presents a uniform thickness of about 0.8 microns. Structurally it seems to consist of a homogeneous basophilic ground substance which, however, does not suggest a fibrous texture.

Stage VI: Because deutogenic and lipogenic activities during this stage become progressively less pronounced, the investing membranes remain in a static condition, except for the intervention of some degenerative changes involving the fibrous connective tissues that lie on the surface of the follicular epithelium and also in the cells of the follicular epithelium. These regressions are manifest shortly before ovulation begins. Nuclei of the fibroblasts of the outer fibrous tunic decrease in size and their morphology is altered so that they often appear as splinter-like elements with respect to which a cytosome could not be identified. The apparent absence of the cytosomes results in a condition in which the naked nuclei seem to lie along the margins of the fibrous elements. The distribution and abundance of the coarse undulating fibers become altered from the previous pattern so that the filaments are often reduced to a single strand that incompletely surrounds the underlying follicular membrane, while at other surfaces the fibers are considerably more numerous. The result of these activities, which are obviously of a degenerative nature, appears to be directed toward a weakening of this particular investment to facilitate the escape of the ovum.

The follicular epithelium evidences a state of deterioration by a transformation of the nuclei from a vesicular form to a dense or pycnotic condition as compared to its Stage V status. Although the cells do not further increase in size, their internal vacuolation becomes more pronounced.

The *zona radiata externa* throughout this stage retains its earlier morphology and tinctorial properties. The *zona radiata vera* on the other hand evidences an increasing basophilia accompanied by a diminution in its thickness. By the time the oocyte has reached maturity the staining response of the *zona radiata vera* becomes more basophilic so that it approximates that of the *zona radiata externa* although of a lesser intensity. A concurrent diminution of the thickness of the sub-zonal membrane of such magnitude that it ultimately is reduced to one-half its previous thickness accompanies the change in its staining properties. Its system of radial canals can no longer be detected due either to their obliteration or to having become obscured due to the increase in the staining intensity of the zonal substances. The significance of the regression of the layer may be interpreted on the basis that having completed its role in bringing the oocyte to maturity it becomes hypotrophied with loss of function, and that the changes are specifically designated to facilitate the es-

cape of the egg by minimizing the restraining capacity of the membrane.

Except for a slight increase in the quantity of the matrix of the plasma membrane, it has undergone no detectable change following its origin during the previous stage. The vitelline membrane, having completed its differentiation during stage VI, lies on the thin layer of faintly acidophilic cortical cytoplasm. No increase has occurred in its thickness, but its margins have attained a greater degree of definition.

Spent Follicle: With the discharge of the ovum, the follicular investments retained in the ovary consist of the fibrous connective tissue components, the cellular layer of the follicle, the *zona radiata vera,* and the *zona radiata externa.* A number of empty follicles are shown in Plate VI, Fig. 19, at a period immediately after ovulation. Separation of the outer fibrous together with the adherent follicular layer from the underlying *zona radiata* occurs either during the rupture of the follicle or immediately thereafter. The composite outer layers are visible in the figure as gray undulating lines. This separation is initiated by localized elevations of the epithelial layer with respect to the underlying *zona radiata externa.* In these areas numerous circumferential clefts arise and disjunction of the membranes occurs. Through an extension of the process, it soon involves all areas of the follicle.

Concurrent with the sloughing off of the follicle epithelium and its transformation into the *corpus albicans,* the *zona radiata externa* diminishes to a thickness of 1 micron or less, and the *zona radiata vera* to 1 to 1.5 microns. Disappearance of the canals which were present when the follicle was functional occurs immediately with egg release. Following a short interval the two layers, the components of which soon become indistinguishable from each other, become further reduced in thickness to less than 1 micron. The few or numerous fractions thus created gradually lose their basophilic properties and finally become achromatic and disappear. Since these changes occurred uniformly in the material, and because of the absence of mediating cells, the responsible enzymes must be elaborated elsewhere in the lamella and be generally distributed throughout its tissues. The absorptive process is very rapid, probably not requiring more than one or two weeks.

The epithelial layer of the follicle becomes materially thinner, a change which is accompanied by, or is the result of, a transformation of its cellular components into a low cuboidal form. The faces of most of these cells which were in contact with the *zona radiata externa* present a shredded aspect, due possibly to the tensions exerted in the separation of the layers. Some nuclear degeneration is indicated by the prevalence of dense or pycnotic types having a mean diameter of 1.3 microns and which are slightly elongated and strongly basophilic. The vacuolated cytoplasm is converted during the initial period of nuclear transformation into a homogeneous mass of moderate density consisting of fine acidophilic granules. Further regression of the follicular epithelial layer and the establishment of a *corpus albicans* are accomplished by a shrinkage of the follicular layer, thereby decreasing its diameter, and an inward extension or

movement of the cells into the central cavity. As the layer moves inward, it is accompanied by the superficial fibrous connective tissue investment which begins to show evidence of regeneration. As a result of the inward extension of the epithelial cells, the central cavity ultimately becomes obliterated and a *corpus albicans* is produced. Subsequently the cellular components appear to undergo a form of regression in which they become dilated and devoid of cytoplasm. Their nuclei shrink in size, assume a pycnotic state, and ultimately disappear. At this period the corpus appears as illustrated in Plate V, Fig. 20.

During the early phases of regression, the connective tissue elements lying on the surface of the body begin to receive numerous vascular buds so that they soon occupy almost the entire peripheral area. A number of these channels can be identified in the figure by the inclusion of the dark nucleated blood cells. As the vascular flow increases, the *corpus albicans* diminishes in size until it completely disappears. It is believed that leucocytic action is responsible for the absorption of its substance although this point was not definitely established.

In the final stages the corpus is reduced to a complex consisting only of the fibrous elements and a few blood vessels. Ultimately the connective tissue becomes very tenuous so that it can not be distinguished from the general stromal material. During this time, the vascular entities decrease in numbers so that they, too, are finally obliterated.

The removal of the *corpus albicans* apparently occurs gradually requiring several weeks for its completion. Residual bodies have been encountered in spent ovaries as late as mid June.

IX. SUMMARY

In the Gulf of Mexico menhaden, *Brevoortia patronus*, events relating to gonadogenesis have not previously been described. In the present study it was found that the process first becomes discernible in 17 to 18 mm larvae with the appearance of small numbers of primordial germ cells in the retroperitoneal dorsal-lateral areas of the body wall. Shortly thereafter, when the fish have attained a length of about 23 mm, the germinal ridge becomes evident. With this development, the germ cells begin a leisurely migration toward the epithelial thickening to become incorporated in the germinal fold as occurs in other vetebrates. This migratory activity also includes a complement of associated body wall mesenchymal or fibroblast elements from which the stromal and connective tissues of the organ will arise. With further substention of the germinal fold, the cells of retroperitoneal origin pass into it. By the time the larvae have attained a length of 29 mm, the gonad has attained its definitive form and is suspended in the coelom by a broad mesentery.

Gonadogenesis was found to occur only in larvae that had arrived in a euryhaline littoral habitat. This relationship makes it appear that the activity is incapable of being initiated in waters of high salin-

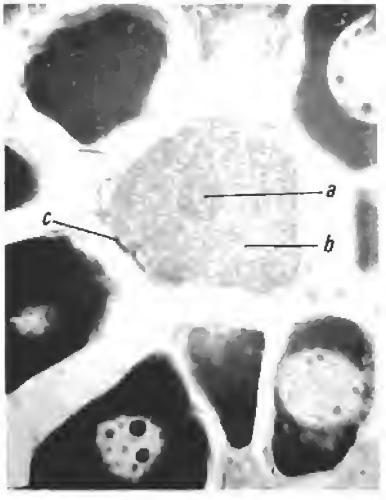


Fig. 22

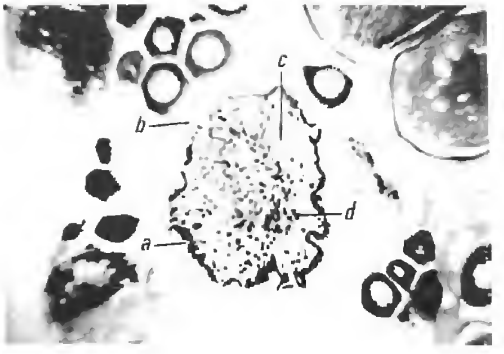


Fig. 24

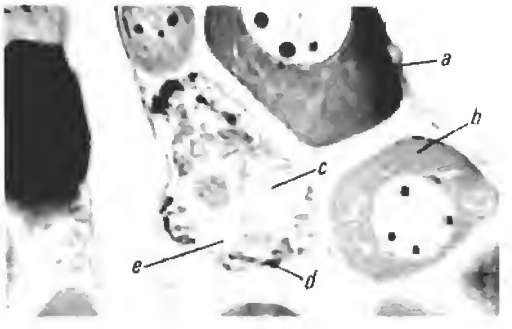


Fig. 25

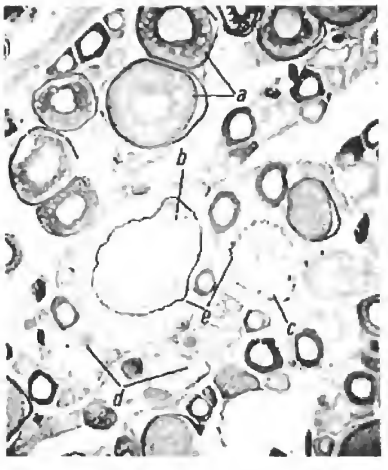


Fig. 23

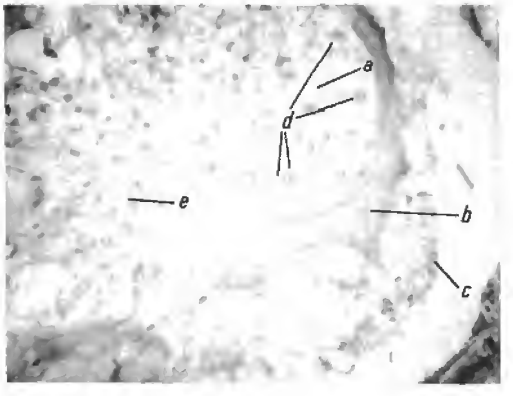


Fig. 26

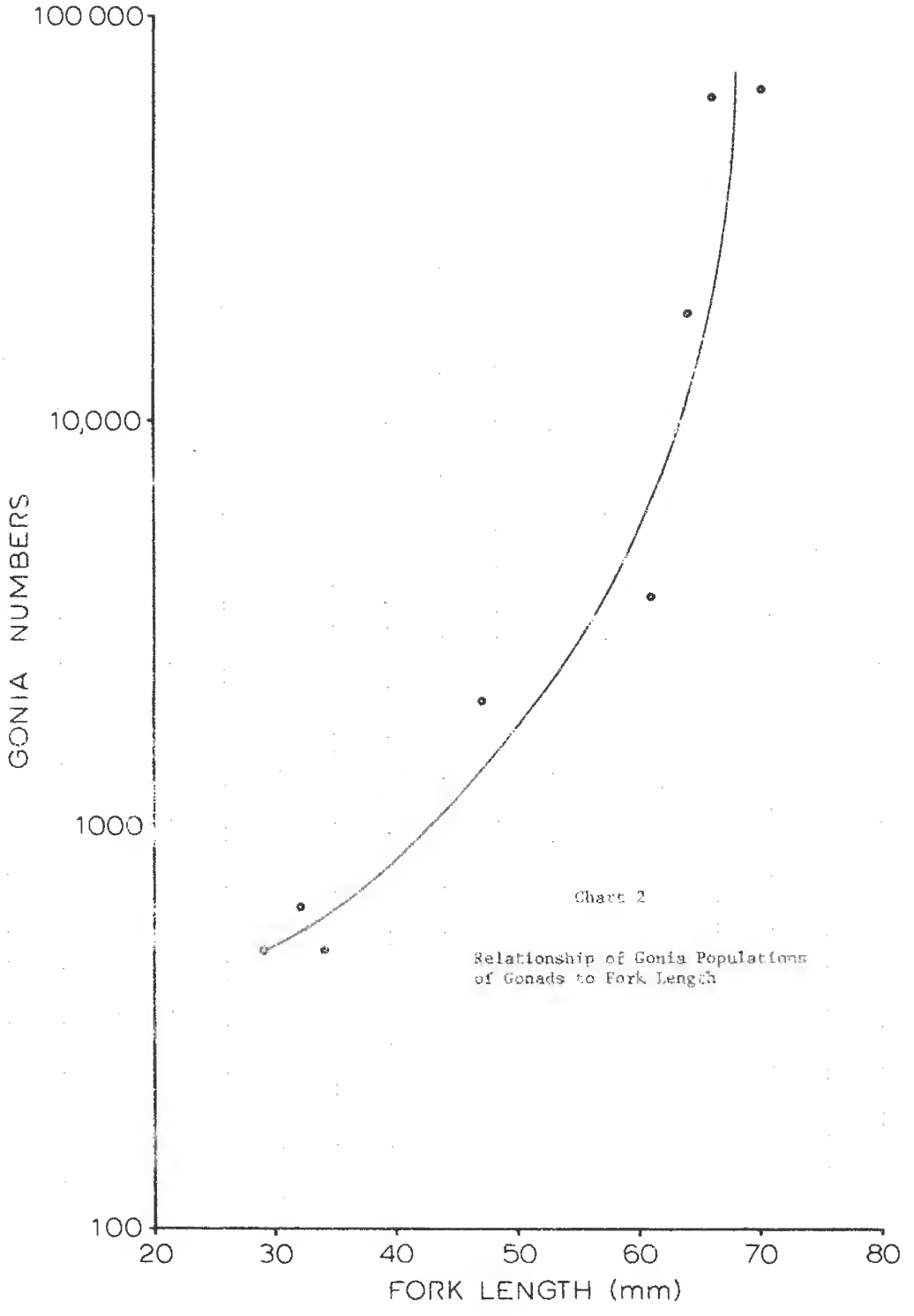


Chart 2

Relationship of Gonia Populations of Gonads to Fork Length

ity. It furthermore appears that only a relatively brief interim is required in the estuarine habitat to induce gonadal inception. This conclusion is derived from the known larval size at the time of their entrance into this environment. Although some variation can be expected regarding the beginning and termination of the period of gonadogenesis due to the intermittency with which the larvae reach the low salinity habitat, it is confined to April and May.

During the remainder of the larval period the organ rapidly increases in size but remains in the primitive state of organization. Its enlargement is in large part due to the intense activity of gonial production. Whereas the number of these cells in the newly formed gonad is less than one hundred, subsequent replication in the nature of geometrical rates of increments provides a population of more than 60,000 by the time the organ has attained a length of 66 mm. Ultimately with the appearance of sexual characteristics in the gonads, the rate of production of new generations in the female is greatly diminished, while in the male it is maintained at a relatively high level. The accelerated growth of the organ and the augmentation of the supply of gonia is most pronounced during the mid- and late summer.

As in other vertebrates, the gonad is histologically indifferent in its earlier stages. Sex differentiation in the material studied had definitely occurred in post-larvae having a length of 60 mm. It is believed, however, that the initial transitional events probably occur in fish of about 50 mm, representatives of which were not available for study. The conclusion that the change occurs in the 50 mm range is supported by the complete absence of the sexual condition in all specimens smaller than this size as related to the established condition in all of the 60 mm fish. Thus the intermediate phases in which evidence of its appearance would be manifest in some post-larvae and not in others were not observed. The earliest characteristics which differentiate the ovary from the testis include its more rotund shape in cross sections, the development of a central and a number of stromal cavities, the heavier connective tissue investment of its gonia, the greater density of its stromal components, the relatively smaller number of gonia present, and the appearance of a number of bays or indentations of the central cavity that foreshadow the development of the ovigerous lamella. The transformation of the indifferent gonad into a primitive ovary in which the pattern of the ovigerous lamella and the arrangement of the gonia become established appears to occupy several weeks. In most of the specimens the process initially became apparent in June, although in others it was delayed until July or the first part of August. The differential between the earlier and later dates is considered to reflect variations in time of hatching and rates of migration of the larvae into the estuarine waters.

Zero year fish are incapable of completing gametogenesis prior to the onset of the ensuing spawning season. Until that time most of the sex cells of the ovary are oogonia or transitional first stage oocytes. Occasional cells, however, reach a phase of maturity that immediately precedes the elaboration of oil. Such oocytes remain in this condition until the beginning of the following cycle or are aborted. The latter condition is not prevalent. Fish evidencing incomplete gametogenesis

do not participate in the annual migration to the spawning grounds in the offshore waters. It is only after mature ova appear in fish having a body length of 100 mm or more that they are encountered during the spawning season in higher salinity environments.

In mature females, the gametogenic cycle has been divided into specific phases or stages by various investigators on the basis of the sequential occurrence of histologic changes in the oocytes. In these cases, there exists considerable variability between the classifications. As regards the clupeids, Naumov (1956) recognizes in the Murmansk herring five phases which, however, in the case of *Brevoortia patronus* appear to be inadequate to account for all the transformations encountered. For this reason a total of six stages are dealt with in the present paper. The earliest stage is the undifferentiated mitotic cell that occurs in variable numbers in all functional gonads. Although Naumov states that these cells are present in the ovary at all times, the present study shows that their numbers are inversely proportional to the degree of maturity of other oocytes in the organ. For a considerable period while most of the oocytes are passing through the final maturational phases, these first stage cells could not be seen in any part of the ovary, although they reappear at the beginning of subsequent cycles. Transformation of these oocytes into the next stage occurs principally at the onset of a new cycle. At this time the author considers them as being in Stage II, which is characterized by cellular and nuclear enlargement accompanied by the development of a cytoplasmic acidophilia. These cells are still capable of mitotic activity. Oocytes which have not advanced beyond the first two stages are capable of being maintained from year to year in a *status quo* condition as evidenced by the non-occurrence of atresia.

The following phase, Stage III, is marked by the intra-nuclear appearance of scores of nucleoli-like bodies that lie against the inner face of the nuclear membrane and which are responsible for lipogenesis and deutogenesis. They exhibit several characteristics that preclude their being ordinary nucleoli including their persistence after the disappearance of the original nucleolus, differences in their behavior during the early stages of atresia, the release of their substance into the cytoplasm, and in the final stages of oocyte maturation, the sudden discharge of large numbers of them into the cytosome concurrently with the absorption of the nuclear membrane, and their specialized role in fat and yolk formation as stated above. Since they induce and regulate the production of metaplasmic materials, they are identified in this study by the terms *proto-vitellonucleoli* and *eu-vitellonucleoli* according to whether they are operating within or outside the nuclear membrane, respectively. The so-called yolk nuclei do not occur in menhaden oocytes. Although maturation activities are sufficiently advanced in this stage that oil production is initiated, they retain the potentiality of remaining in the ovary from one cycle to the next without undergoing atresia.

After oil production has been initiated as described above the oocyte becomes radically altered as regards both the nucleus and the cytosome. These changes reflect functional adjustments in the oocyte that presage the onset of yolk formation. The cells at this time are in an early phase of Stage IV. During the later phases provisional

yolk will make its appearance. Oocytes which have reached this stage of maturation have lost all potentialities to continue to reside in the ovary from season to season and must complete their development prior to spawning or be aborted. The length of the period that they remain at this level of maturation is short, probably not exceeding a few days. They normally begin to appear in ovaries during October. From that time until their disappearance in January they represent about 24% of the total number of all oocytes in the organ.

The fifth maturational stage is principally devoted to yolk formation although some oil continues to be produced during this period. At about mid-phase, the prominent nucleus shifts to a somewhat eccentric position in the cytosome as the peripheral oil globules begin migrating to the center of the cell. The displacement of the nucleus marks the beginning of its dissolution. This event is characterized by the absorption of the nuclear membrane and the discharge of its contents into the cytosome. Included in the effluent material are hundreds of *proto-vitellonucleoli* of many sizes. In the areas where nuclear and cytoplasmic matrices are confluent, these bodies are transformed, either directly or by a series of indistinguishable metabolic changes into yolk granules which are indistinguishable from that produced earlier. At the close of the fifth stage no visible components of the nucleus remain. Although in occasional instances a small number of oocytes at this phase of maturation occur in ovaries as early as October and as late as March, they reach a modal point in December. In January, their population is about one-half of the December numbers.

In the absence of a nuclear control mechanism, the final stage of maturation of the oocyte is limited to the completion of activities that had their origin in the previous stages. A residual migration of the oil globules ultimately brings these bodies together to constitute a large centrally located mass in which the separate components undergo a degree of coalescence. The yolk granules show evidence of undergoing changes as indicated by a modfication in their staining properties. These modifications give rise to a condition in which some confluence develops between the separate granules. Many of the oocytes complete these events in advance of other cells and are retained in a "resting" state until the initiating of spawning activities.

The histology and cytology of the oocyte follicle is similar to that encountered in other members of the Clupeidae. The structure arises during the third stage of maturation through the application of a small number of fibroblast cells from the adjacent lamellar stroma to the surface of the oocyte. Enlargement of the follicle during the ensuing stages of oocyte maturation is accomplished by the mitotic activity of the original investing fibroblasts rather than by the introduction of additional stromal cells. The original squamous form of the investment progressively becomes transformed into a simple cuboidal condition. During the period of this transition and prior to the elaboration of yolk, the follicle lays down the *zona radiata*. Although frequent references occur in the literature describing it as having a uniform structure throughout, the present study shows that in the Gulf menhaden it consists of outer and inner layers which possess different staining properties. The external layer has been designated

in this study as the *zona radiata externa* while the internal division is appropriately referred to as the *zona radiata vera*. Both layers are permeated by the *zonal* canals. The functional significance of each of the layers, other than serving as an exchange mechanism, is not known.

Atresia is not uncommon in menhaden oocytes although the activity is limited to the phases of maturation following the introduction of lipogenesis, i. e. Stages III through VI. Although certain ovaries may show a rather high rate of atresia, the condition of mass mortality was never observed. It most commonly occurs from early fall to late winter and is associated with the crowding of the oocytes in the organ, and in the case of spent ovaries, the removal of yolk laden oocytes which were not spawned. In a few aberrant cases considerable atresia was found in yolk containing cells prior to the beginning of the running period. It appears in this situation that some form of hormonal imbalance is the precipitating factor. While considerable controversy exists as to the nature of the mechanism involved in the removal of the atretic cells, the evidence provided in this study shows that the follicle cells are implicated. This is particularly noticeable in the case of atretic yolk bearing oocytes which are invaded by numbers of cells of follicular origin. The chemical degradation of the detritus and its ultimate removal is brought about by extra-cellular enzymes produced by the transformed follicle cells. With the establishment of vascular elements at the periphery of the detritus during the terminal phases of the absorptive process, the cells which have been responsible for the digestion of the debris disappear and a few leucocytes appear in the residuum. Evidence suggests that the disappearance of the lytic cells is brought about by their de-differentiation into fibroblasts and incorporation into the connective tissues of the lamella.

Earlier studies have indicated that spawning of *Brevoortia patronus* in the Gulf of Mexico occurs during the winter months. This is confirmed by the present work in which it is shown that the ovaries contain ripe oocytes from October to March. Although the study did not delineate the exact geographical areas in which the activity occurs, it provides evidence that it transpires only in high salinity environments.

Spawning is initiated in one year fish, irrespective of the seasonal period that catches were made. Ovaries taken from females of less than 100 mm fork length contained only the earliest stages of oocytes. These have been shown to be stable and capable of being carried over from year to year.

Spawning in this species is total but intermittent. At the close of the running season the organ is depleted of mature oocytes. The intermittent nature of the activity is established by the continued presence over a period of months of spawnable oocytes together with numbers of advanced stages which are potentially spawnable. Beginning with October, the earliest date that spawnable eggs are present in the ovaries, the potentially spawnable oocytes (Stages IV and V) concurrently present range from 8% in that month to a mode of 28% in November, thereafter decreasing to 9% in February. Therefore spawning is not only intermittent, but the process occupies an extended period.

X. LITERATURE CITED

ARNOLD, EDGAR L., JR.

1958. Age and growth of menhaden. Annual Report Gulf Fisheries Investigation, Fish and Wildlife Service, United States Department of the Interior, Circular 62: 59-60.

BALFOUR, F. M.

1878. Development of elasmobranch fishes. Chapter VI.

BARFURTH, D.

1886. Giologische untersuchungen uber die Bachforelle. Archiv für Mikroskopische Anatomie, Bd. 27: 128-179.

BOLOGNARI, ARTURO

1958. Osservazioni al microscopic elettronico sulla formazione del vitello negli ovociti de *Patella coerulea* L. con qualche considerazione sulle granulazione ribonucleoproteiche del citoplasma e sulle apparato del Golgi. Bollettino di Zoologia, 25: 155-169.

CLARK, FRANCES N.

1934. Maturity of the California sardine *(Sardina caerulea)*, determined by ova diameter measurements. Division of Fish and Game of California, Bureau of Commercial Fisheries (California), Fish Bulletin 42, Contribution 119, 1-52.

CRAIG-BENNETT, A.

1930. The reproductive cycle of the three spined stickleback, *Gasterosteus aculeatus* Linn. Philosophical Transactions of the Royal Society of London, Series B, 219: 197-279.

CUNNINGHAM, J. T.

1894. The ovaries of fishes. Journal of the Marine Biological Association of the United Kingdom 3(2): 154-165.

1898. On the histology of ovarian ova of certain marine fishes. Quarterly Journal of Microscopical Science. 40: 101-163.

GATENBY, J. B.

1922. Gametogenesis of *Saccocirrus*. Quarterly Journal of Microscopical Science. 66: 74-96.

GATENBY, J. B. and J. H. WOODGER

1920. On the relationship between the formation of yolk and the mitochondria and the Golgi apparatus during oogenesis. Journal of the Royal Microscopical Society. : p. 129.

GHOST, ASOK and AMIYA B. KAR.

1952. Seasonal changes in the gonads of the common Indian catfish *(Heteropneustes fossilis* Bloch). Proceedings of the Royal Society of Bengal. 5(1): 29-50.

HICKLING, C. F.

1935. Seasonal changes in the ovary of the immature hake, *Merluccius merluccius* L. Journal of the Marine Biological Association of the United Kingdom. 20: 443-461.

HICKLING, C. F. and E. RUTENBERG.

1936. The ovary as an indicator of the spawning period in fishes. Journal of Marine Biological Association of the United Kingdom. 21(1): 311-317.

HIS, W.

1873. Untersuchungen uber das Ei und Eiertwicklung bei Knochenfischen. Leipzig.

JAMES, MARIAN F.

1946. Histology of gonadal changes in the bluegill, *Lepomis macrochirus* Rafinesque, and the large mouth bass, *Huro salmoides* (Lacépède). Journal of Morphology 79: 63-92.

KUNTZ, ALBERT and LEWIS RADCLIFFE.

1917. Notes on the embryology and larval development of twelve teleostean fishes. Bulletin of the U. S. Bureau of Fisheries, 35: 87-134.

MacLEOD, J.

1881. Recherches sur la structure et la dévellopement de l'appareil reproducteur femelle des teleostéens. Archives de Biologie, 2: 497-518.

MALONE, THOMAS E. and K. KENNETH HISAOKA.

1961. The formation of deutoplasmic components in the zebrafish ovary. American Zoologist 1(3): 371-372.

MENDOZA, G.

1939. The reproductive cycle of the viviparous teleost, *Neotoca bilineata*, a member of the family Goodeidae. IV. The germinal Tissue. Biological Bulletin 76: 87-97.

1939. The reproductive cycle of the viviparous teleost, *Neotoca bilineata*, a member of the family Goodeidae. II. The cycle changes in the ovarian soma during gestation. Biological Bulletin 78: 349-365.

MOORE, G. A.

1937. Germ cells of trout *(Salmo irideus* Gibbons). Transactions of the American Microscopical Society, 56(1): 105-112.

NAGASAKI, FUZUKO

1958. The fecundity of Pacific herring *(Clupea pallasi)* in British Columbia Coastal Waters. Journal of the Fisheries Research Board of Canada, 15(3): 313-330.

NAUMOV, V. M.

1956. The herring of the North European Basin and adjacent seas. (Ovogenesis and ecology of the sexual cycle of the Murmansk herring *(Clupea h. harengus* L.). U. S. Fish and Wildlife Service Special Scientific Report-Fisheries No. 327 (Translation 1959) pp. 203-261.

NELSEN, M. A.

1953. Comparative Embryology of the Vertebrates. Blakiston Co., New York, 1-982 pp.

RABL, C.

1896. Ueber die Entwickelung das urogenital systems der Salachier. Morphologische Jahrbucher, 24: 632-767.

REINTJES, J. W.

1961. Menhaden eggs and larva from M/V Theodore N. Gill cruises South Atlantic Coast of the United States. 1953-1954. U. S. Fish and Wildlife Service Special Scientific Report—Fisheries No. 393, pp. 1-7.

1962. Development of eggs and yolk-sac larvae of yellowfin menhaden. U. S. Fish and Wildlife Service Fishery Bulletin 202, 62: 93-102.

SEMPER, C.

1875. Das urogenital system der Plagiostomum und seine Bedeutung für das der Wirbeltheire. Arbeit Zoologie. Zoologische Institut der Wurzburg. Bd. II, pp. 195-509.

STUHLMANN, F. L.

1887. Zur kenntnis des ovariums der Aalmutter *(Zoarces viviparus* Guv.) Abhandlungen and Verhandlungen des Naturwissenschaftlichen Vereins in Hamburg, 10: 1-36.

SUTTKUS, ROYAL D.

1956. Early life history of the Gulf menhaden, *Brevoortia patronus,* in Louisiana. Transactions of the Twenty-first North American Wildlife Conference, pp. 390-406.

SUTTKUS, ROYAL D. and BANGALORE I. SUNDARARAJ

1961. Fecundity and reproduction in the large scale menhaden, *Brevoortia patronus* Goode. Tulane Studies in Zoology 8(6): 177-182.

WALLACE, W.

1903. Observations on ovarian ova and follicles in certain teleotean and elasmobranch fishes. Quartely Journal of Microscopical Science 47 (2): 161-213.

WHEELER, J. F. G.

1924. The growth of the egg in the Dab *(Pleuronectes limanda)*. Quarterly Journal of Microscopical Science, N. S. No. 272, (London), 68: 47-81.

WOODS, F. A.

1902. Origin and migration of the germ cells in *Acanthias*. American Journal of Anatomy 1: 307-320.

YAMAMOTO, KUCHIRO

1958. Studies on the formation of fish eggs. XII. On the non-massed yolk in the egg of the herring, *Clupea pallasii*. Bulletin of the Faculty of Fisheries, Hokkaido University, Japan, 8(4): 270-275.

XI. EXPLANATION OF PLATES

PLATE I

Fig. 1. Area of gonad formation in an 18 mm larva. The body wall is separated from the gut by the diagonal clear area. Lying above the band of melanophores are two primitive germ cells, a number of fibroblasts, and a quantity of aerolated connective tissue. The paerital epithelium that occurs on the coelomic face of the melanophores is not visible in the figure, but its position coincides with the numerous small pigment granules that have been discharged by the melanophores. A fibroblast is seen entering the interstice between two pigment cells. (FeH & E x.s; 15x-90x obj.) a. Germ cell, b. Parietal epithelium, c. Melanophores, d. Coelom, e. Fibroblasts, f. Pigment granules, g. Viscera.

Fig. 2. Area of gonad formation from a 24 mm larva showing the origin of the germinal ridge and the aggregation of retroperitoneal cellular elements that will soon pass through the spaces between the melanophores so as to be incorporated in the gonad as the epithelium continues to distend into the coelom. Three primitive germ cells occur in the upper area of the retroperitoneal cellular complex. (FeH & E, x.s; 10x-90x obj.) a. Germ cells, b. Germinal epithelium, c. Parietal epithelium, d. Melanophores, e. Fibroblasts, f. Coelom, g. Gut.

Fig. 3. Primitive gonad from a 29 mm fish shortly after the subtention of the germinal ridge and the entrance of the retroperitoneal elements. A single centrally located oogonium is enclosed in a capsule of circularly arranged fibroblasts. The organ at this stage has a robust profile and a relatively broad mesentery. (Cajal; x.s; 12x-43x obj.). a. Germ cell, b. Capsular layer, c. Stroma, d. Gut, e. Blood cells, f. Gonadal epithelium, g. Coelomic layers of body wall, h. Coelom.

Fig. 4. Older gonad from a 47 mm fish showing the precocious origin of the central cavity and numerous spaces. These features are characteristic of the metamorphosis of the indifferent gonad into an ovary. A number of spherical oogonia occur among the stromal fibroblasts. The form of the organ is less robust than earlier. (Cajal; x.s; 5x-43x obj.). a. Germ cells, b. Central cavity, c. Stromal cavities, d. Gut, e. Coelomic layers of body wall, f. Coelom, g. Air Bladder.

Fig. 5. Ovary from a 70 mm fish showing enlargement of the central ovigerous cavity. The oviducal space lies at the upper left. The stromal spaces are more prominent than earlier. The rate of replication of the oogonia is evident by comparison with Fig. 4 (FeH & E; x.s; 10x-10x). a. Germ cells, b. Stroma, c. Central cavity, d. Stromal cavities.

PLATE II

Fig. 6. Ovary from an 84 mm fish caught in August. The radial indentations leading from the central cavity into the stroma,

which was slightly foreshadowed in Figure 5, is well underway. These transverse clefts represent the manner by which the stroma becomes divided to give rise to the ovigerous lamella. The inter-ovigerous clefts become continuous with the stromal spaces which existed earlier so as to extend them radially. The visible germ cells are in the earliest stages of maturation. Oogonia are not visible. (FeH & E; sag. sec; 12x-10x obj.). a. Inter-ovigerous clefts, b. Central cavity, c. Oocyte transitional from Stage I to Stage II, d. Stromal cavity, e. Stage I Oocyte.

Fig. 7. A 14 mm ovary from an adolescent fish showing the fundamental early form of the ovigerous lamellae before their subsequent modifications in which they become tortuous, subdivided, and of unequal thickness due to the burden of the maturing oocytes. The paucity of the intra-lamellar connective tissue characteristic of these structures is evident. (Cajal; x.s; 10x-43x obj.). a. Three Stage I oocytes entering Stage II, b. Inter-ovigerous clefts, c. Intra-lamellar connective tissue, d. Early Stage III oocytes, e. Stage II oocytes.

Fig. 8. Detail of the *tunica albuginea* of a 26 mm ovary from a female caught in September. The morphology of the intra-lamellar septum as it arises from the *tunica albuginea* is shown. The tunic, here seen as a broad vertical band of tissue, consists of four layers at this age, the two internal layers of which are poorly separated from each other. From right to left they are a thin reflection of the visceral epithelium; a fibrous layer which has been cut transversely in the figure so that the fibers appear as almost colorless dots and among which numerous dark fibroblasts occur; a layer of darkly staining smooth muscle; and an internal investing layer of epithelial-like cells. The latter is shown at the bottom extent of the tunic as a light grey amorphous band. (Flemming; l.s, 12x-43x obj.). a. Visceral epithelium, b. Circular fibrous layer, c. Smooth muscle, d. Position of internal layer of epithelial-like cells, e. Intra-lamellar septum.

Fig. 9. Adult ovary from a fish caught in May. Two nests containing a total of seven Stage I oocytes are represented. The dark nucleolus is centrally located within the large nucleus. The thick serrate walled condition of the nucleus is due to the peripheral deposition of chromatin. The cytoplasm is achromatic. Cells at this stage typically occur near the margin of the lamellae and lack a well organized capsule or follicle investment. (FeH & E; x.s; 15x-90x obj.). a. Inter-lamellar cavity, b. Nests of Stage I oocytes, c. Connective tissue associated with nests.

PLATE III

Fig. 10. A composite figure showing three Stage II oocytes taken at a magnification slightly less than Figure 5. A number of these cells are also visible in Figure 7 where they appear as having large clear nuclei surrounded by a thin grey layer of cytoplasm. The cytosome at this time has developed a moderate eosinophilia that makes it more evident than earlier. The nucleus has assumed a greater prominence and encloses

varying amounts of chromatin or occasionally clearly defined chromosomes if the cells are mitotically active. (FeH & E; x.s; 12x-90x obj.). a. Stage I oocytes, b. Inter-lamellar cavity, c. Typical Stage II oocyte, d. Late Stage II oocyte.

Fig. 11. A group of Stage III oocytes showing their characteristic angular shapes. The darkness of the cytosomes is due to the extreme basophilia which occurs at this time. The large cell at the right contains within its nucleus the remnant of the nucleolus. Numbers of the *proto-vitellonucleoli* are arranged around the inner face of the nuclear membrane. These oocytes are present in all mature ovaries throughout the year. (Flemming; x.s; 15x-10x obj.). a. *Proto-vitellonucleoli,* b. Remnant of nucleolus.

Fig. 12. An early phase of Stage III oocyte. The slight cytosomal basophilia which occurs throughout is being replaced by an intense basophilia which is initiated and controlled by the *proto-vitellonucleoli.* It will be observed that the points of origin of the basophilic strands co-incide with the distribution of the *proto-vitellonucleoli.* Ultimately the strands become confluent giving rise to the state shown in Figure 11. (FeH & E; x.s; 10x-43x obj.). a. Basophilic strands, b. Fat globule, c. *Proto-vitellonucleoli,* d. Shrunken mass of karyoplasm, e.Eosinophilic cytoplasm.

Fig. 13. An early Stage IV oocyte showing the loss of peripheral basophilia, the appearance of provisional yolk granules (which imparts a granular aspect to the cytosome), and continued formation and coalescence of the oil globules. The *proto-vitellonucleoli* continue to increase in numbers. The thin black line lying at the margin of the oocyte represents the inception of the *zona radiata.* (Flemming; x.s; 5x-43x obj.). a. Region of provisional yolk granules, b. Aggregations of oil globules, c. Layer of *proto-vitellonucleoli,* d. Developing *zona radiata.*

PLATE IV

Fig. 14. A typical late Stage V oocyte. The nucleus has assumed an eccentric position and its former location is becoming occupied by a central oil body. Considerable discontinuance of the peripheral and central oil globules also occurs at this period. The nuclear membrane is in the process of regression and the nuclear matrix contains great numbers of the final generations of the *proto-vitellonucleoli.* The epithelial follicular investment is well-developed, but only slightly visible in the figure, while the *zona radiata* appears quite distinctly as a homogeneous circular band. (FeH & E; x.s; 10x-10x obj.). a. Nucleus, b. Oil body, c. *Zona radiata,* d. Definitive yolk, e. Residual *proto-vitellonucleoli.*

Fig. 15. Detail of the nucleus and its environs of a late Stage V oocyte. The *proto-vitellonucleoli* have been discharged and the nucleus is in the process of freeing the *eu-vitellonucleoli* into the cytosome which is now confluent with the nuclear matrix.

The extruded *vitellonucleoli* appear to be capable of being directly transformed into yolk. This is shown in the figure by the gradual transition in size of the bodies as they approach the cytosomal area so that no distinction can be made between the yolk and the *vitellonucleoli.* Following the liberation of these bodies, the nuclear matrix becomes incorporated in the cytosome and it is lost from view. (FeH & E; x.s; 10x-43x obj.). a. Karyoplasm, b. *Eu-vitellonucleoli,* c. *Proto-vitellonucleoli,* d. Oil globules, e. Layer of provisional yolk, f. Definitive yolk.

Fig. 16. An early stage VI oocyte shortly after the absorption of the nucleus illustrating the form of the central oil mass and the chromophobia of the yolk which develops at this time. With some further coalescence of the components of the central oil mass, the oocyte is in a condition for spawning. The follicular investments remain substantially the same as in Stage V. (FeH & E; x.s; 12x-10x obj.).

Fig. 17. A composite figure showing the origin of the follicle. Its inception involves the application of a few fibroblasts to widely separated areas of the surface of the oocyte. The Stage III oocyte at the upper right shows the condition slightly after the initiation of the activity. In this case about seven primitive follicle cells lie on the surface of the oocyte. The oocytes at the bottom and upper right are in early Stage IV. They are now completely covered by a follicular investment from which additional investing cells will be derived by mitosis. The *zona radiata* is represented by a dark line. (Cajal; x.s; 15x-43x obj.). a. Fibroblasts, b. *Zona radiata.*

PLATE V

Fig. 18. A composite view of Stage V follicles showing the characteristic cuboidal nature of the epithelium and its outer fibrous connective tissue investment. Lying under the epithelial covering is the *zona radiata* which, in the case of the oocyte at the lower left, shows its typical differentiation into an outer *zona radiata externa* and an inner *zona radiata vera.* A suggestion of the presence of the radial canals which penetrate this structure is visible. Under the zonal structures lies the vitelline membrane which in the figures shows a detachment from the zonal investment. The follicle remains in this condition until spawning. (FeH & E; x.s; 15x-90x obj.). a. Vitelline membrane, b. Epithelial layer, c. *Zona radiata externa,* d. *Zona radiata vera.*

Fig. 19. A number of follicles shortly after spawning. The *zona radiata* (black margins) enclose a follicular fluid which has formed an amorphous precipitate due to the techniques used in preparing the sections. External to the *zona radiata* the grey wavy lines represent the remains of the epithelial portion of the follicle. The darker cells at the bottom and to right are Stage III and Stage IV oocytes that show evidence of atresia. (FeH & E; x.s; 10x-10x obj.). a. *Zona radiata,* b. Epithelial follicular investment.

Fig. 20. An early stage in the absoption of a spawned follicle. The vascular system soon invades the follicle wall as shown by the numerous channels containing hemal elements at the periphery of the follicle. With the removal of the internal coagulum shown, the follicular layer will move inward folding upon itself meanwhile. In this manner it will give rise to the *corpus albicans* which will ultimately during its regression contribute its cells to the stroma in the form of fibroblasts. (FeH & E; x.s; 12x90x obj.). a. Vascular channels, b. Regressing *zona radiata,* c. Hypertrophied fat globules, d. Degenerating yolk granules, e. Intra-lamellar fibroblasts.

Fig. 21. An early phase of atresia of a Stage III oocyte. While this epoch has many manifestations, it is usually accompanied by an exaggerated enlargement of the nucleolus with disruption of its morphology, and the concurrent outward migration of the *proto-vitellonucleoli* after they have become confluent with each other. Cytosomal basophilia is materially reduced with the onset of the condition and the cytoplasm becomes noticeably granular. The dark peripheral masses apparently represent changes in the *zona radiata* which becomes first visible during the later phases of this stage. They are not always present. (FeH & E; x.s; 15x-43x obj.). a. Confluent *proto-vitellonucleoli,* b. Hypertrophied nucleolus, c. *Zona radiata,* d. Granulating cytoplasm.

PLATE VI

Fig. 22. A later phase of a Stage III oocyte in atresia. The nuclear components are the first elements in the cell to disappear. In the figure a remnant of the nucleolus remains. The cell continues to decrease in size until it can no longer be seen. The cells of the partially formed follicle return to the stroma as fibroblasts. (FeH & E; x.s; 15x43x obj.). a. Nucleolar remnant, b. Granulated cytoplasm, c. *Zona radiata.*

Fig. 23. A view of several Stage V oocytes undergoing atresia. The earliest signs of regression in cells of this age is a sudden dilation of the nucleus (not shown) and its almost immediate disappearance as seen in the two oocytes in the center of the picture. Concurrently the *zona radiata* separates from the overlying cellular follicle and irregularly begins to sink into the cytosome. There it later segments as shown in the oocyte at center right. Immediately under the central large oocyte, and also in the upper right hand area of the figure can be seen the remnants of two cells which are reaching the terminal phase of reabsorption. (FeH & E; x.s; 5x-10x obj.). a. Normal oocytes, b. Early phase of regression, c. Later phase, d. Oocytes undergoing terminal absorption, e. *Zona radiata.*

Fig. 24. A more detailed view of a Stage V oocyte in atresia showing the characteristic manner in which the *zona radiata* folds upon itself as it sinks deeper in the cytoplasm. A slight fragmentation of the structure is evident. With the movement of the *zona radiata* into the interior of the oocyte, the layers of

follicle cells are brought into juxtaposition with the surface of the oocyte. Until this is accomplished, absorption of the oocyte cannot occur. (Flemming; x.s; 15x-10x obj.). a. *Zona radiata,* b. Follicle layer, c. Aggregation of fat globules, d. Yolk granules.

Fig. 25. A partially absorbed Stage V oocyte. The *zona radiata* has become discontinuous in numerous places as result of the action of digestive enzymes. Similarly the yolk is transformed from a granular state to a somewhat amorphous matrix that has affinity for stains. The fat globules are disrupted so that the material lies free among the yolk. (FeH & E; x.s; 15x-10x obj.). a. Normal late Stage III oocyte, b. Late Stage II oocyte, c. Yolk becoming amorphous, d. *Zona radiata,* e. Unorganized fat.

Fig. 26. Detail of a portion of a Stage V oocyte in about the same stage of absorption as shown in Figure 25. Some evidence of the layer of marginal follicle cells is visible at the bottom of the photograph. Two large amorphic segments of the *zona radiata* occupy a more internal position. Large numbers of small spherical cells occur throughout the internum. These bodies originated from the follicle and are producing extracellular digestive enzymes essential to the removal of the oocyte detritus. The gray irregular masses of yolk evidence the lytic action of enzymes, while the oil (lighter circular areas) is affected to a lesser degree. (FeH & E; x.s; 15x-43x obj.). a. Amorphous yolk material, b. *Zona radiata, c.* Cellular layer of follicle, d. Invading cellular elements, e. Fat mass.

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A Study of *Syngnathus scovelli* in Fresh Waters of Louisiana and Salt Waters of Mississippi

Edward Caldwell Whatley
Gulf Coast Research Laboratory

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A STUDY OF *SYNGNATHUS SCOVELLI*

In

FRESH WATERS OF LOUISIANA

and

SALT WATERS OF MISSISSIPPI

by

Edward Caldwell Whatley

Gulf Coast Research Laboratory
Ocean Springs, Mississippi
and
Department of Zoology, Mississippi State University
State College, Mississippi

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ABSTRACT

A breeding population of *Syngnathus scovelli* was discovered in 1960 in Lake St. John near Ferriday, Louisiana, which is over 300 river miles from the Gulf of Mexico by the shortest possible route. Although *S. scovelli* has been known to be euryhaline, this constitutes the first record of a breeding population in fresh water.

This study encompassed the period from July 1960 through February 1966. During this time *S. scovelli* were maintained for varying periods of time in fresh water aquaria. The longest period of time any one specimen lived in captivity was from 29 September 1960 until 16 November 1962, almost 27 months. The chief limiting factor to the maintenance of *S. scovelli* in fresh water aquaria appears to be a ready supply of live plankton for food.

The breeding process of brackish water specimens was observed and is described. Gestation took 12 days in two males in August and both males bred the day after giving birth to a previous brood.

Young *S. scovelli* measured 12 mm at birth. This is the first report of the length of newly born *S. scovelli* in the literature. A size range of 12-160 mm in length was noted for the species.

A very rapid growth rate has been noted in the young from 12-80 mm, but growth slowed greatly at 80 mm. Three apparent year classes have been postulated among the specimens of *S. scovelli* collected in Lake St. John. These were: 0 (12-80 mm), 1 (80-120 mm), and 2 (120-160 mm). Consecutive monthly data have not been consistent enough to prove this. Failure to collect these data may be due in great measure to the rotenone placed in Lake St. John by the Louisiana Wild Life and Fisheries Commission on 12 October 1961.

The fresh water population from Lake St. John showed less variation in meristic characters than did the fish from Mississippi Sound.

INTRODUCTION

The first of many pipefish, *Syngnathus scovelli* (Plates I and II) was collected by me from fresh water in Lake St. John (Plate III, Figs. 1 and 2) near Ferriday, Louisiana, on 23 July 1960. A cast net, being used in a futile attempt to secure shad for bass bait, was thrown around a bit of naiad *(Najas guadalupensis)*. When the net was shaken out upon the boat seat, the catch yielded a pipefish. As the mesh of the cast net was fairly large (½ inch) and the pipefish small (60 mm long by less that 3 mm in width), this capture was the result of the entanglement of the fish in the naiad.

Lake St. John is an oxbow lake of the Mississippi River which, according to Lambou (1961), was cut off from the Mississippi River by a levee sometime prior to 1879. It is located in Concordia Parish with its extreme northern shore forming the boundary between Concordia and Tensas Parishes. Lambou (1961) states that Lake St. John has a maximum depth of 26 feet, with the majority of the lake ranging from 10-20 feet in depth, a shore line of 17 miles and a surface

area of 2,074 acres. Hutchinson (1957) classified lakes as to their origin and described the processes involved in their formation. Lake St. John was formed by fluviatile action and is classified as type 55 of Hutchinson—an oxbow or isolated loop of meanders.

Gunter (1952) states that levee construction along the Mississippi River started in 1717 at New Orleans and was a gradual process up until about 1880. From that time on the rate was accelerated until the nineteen-thirties when the whole system was greatly extended and more or less stabilized following the disastrous flood of 1927.

A map (Fisk, 1944, Consultant's report: Geological Investigation Mississippi River Alluvial Valley Stream Courses. Mississippi River Commission Vicksburg, File No. MRC/258 85H18C), supplied to the writer by Dr. R. R. Priddy of the Gulf Coast Research Laboratory, shows that an old Mississippi River bed crossed the present site of Lake St. John twice in fairly recent geological time, once 2,000 years ago and again about 1,000 years ago. Pipefish could have become established in this area as far back as 1,000 or 2,000 years ago or within recent years. Today they may swim up the Atchafalaya, Red, Black, and Tensas Rivers (Plate III, Fig. 1) and reach the area through streams overflowing in the spring. It has been shown that certain euryhaline marine fishes ascend the Mississippi River system as far as the Black River (Gunter 1938). The pipefish, *S. scovelli*, has been recorded as euryhaline from several sources and it was not too surprising to find a population in Lake St. John. However, the discovery that this was a resident, breeding population was totally unexpected, for *S. scovelli* is marine and was only known heretofore to breed in salt water. The literature on *S. scovelli* has been largely confined to morphological features and range, with few ecological notes.

Several questions arose as a result of the discovery of *S. scovelli* in the inland fresh waters of Louisiana, namely: (1) was this fish representative of the population previously designated as *S. scovelli?* (2) was this population homogenous? (3) did similar populations exist in other oxbow lakes of the Mississippi River? (4) could this fish be maintained in fresh water aquaria? (5) how long had this population existed in Lake St. John? (6) what is the life history of this fish?

REVIEW OF THE LITERATURE

Jordan and Evermann (1896) indicate that *Syngnathus* was first used in the literature in 1738 by Artedi who published *Syngnathus* in reference to *ophidion, acus, typhle,* etc. in Genera. Linnaeus (1758) through publishing this work of Artedi established *Syngnathus* as a valid genus. Thus Myers (1964) is supported in his statement "There is some reason to believe that the somewhat elder Artedi was largely responsible for the younger Linnaeus' ideas and systems of biological classification." *Syngnathus scovelli* was originally listed as *Siphostoma fuscum* var. in 1894 by Evermann and Kendall. These same authors published a "Description of a new species of pipefish *Siphostoma scovelli* from Texas" in the Proceedings of the United States National Museum, Vol. XVIII, No. 1043, Pages 113-115 1898 (1896).

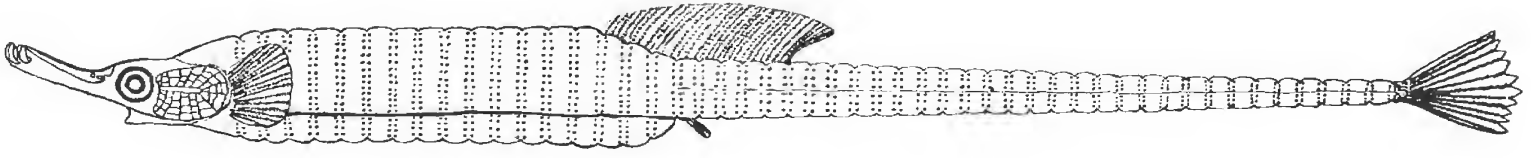


Fig. 1 Female Syngnathus scovelli
(Evermann and Kendall) 1896 Length 147 mm.

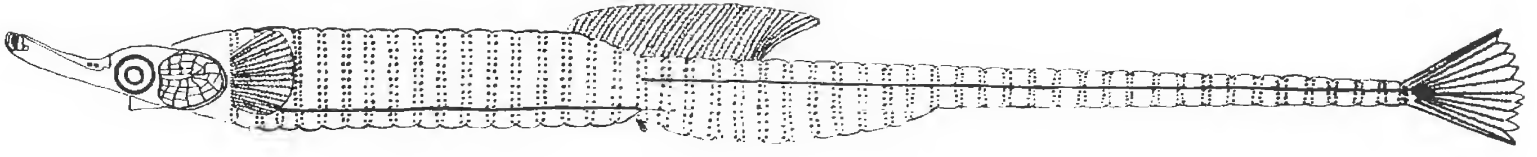


Fig. 2 Male Syngnathus scovelli
(Evermann and Kendall) 1896 Length 147 mm.

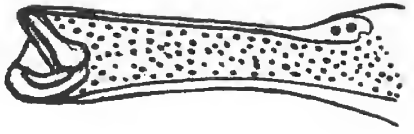


Fig. 1 snout

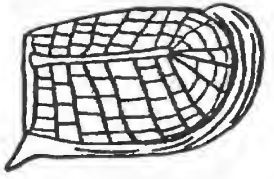


Fig. 2 operculum

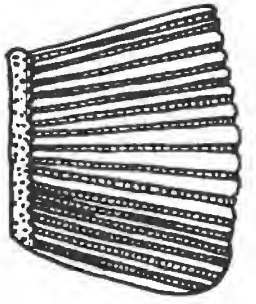


Fig. 3 pectoral fin

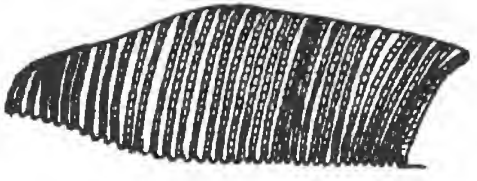


Fig. 4 dorsal fin

Fig. 5 anal fin

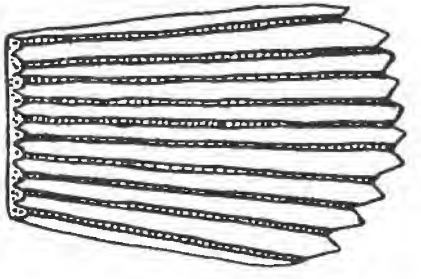


Fig. 6 caudal fin

This reference is basic to the present paper; therefore, it is given in its entirety:

> A re-examination of the specimens of pipefish from Corpus Christi which we referred, with hesitation in an earlier paper, to *Siphostoma fuscum* (Storer), has convinced us that they cannot belong to that species but represent a species hitherto undescribed.
>
> Type—Male and female, No. 47300, U. S. N. M.
>
> Locality—Shamrock Point, Corpus Christi, Texas, where 130 specimens were obtained November 29, 1891, by Messrs. Evermann, Scovell, and Gurley, of the U. S. Fish Commission.
>
> Allied to *Siphostoma affine* (Günther).
>
> Description of female—Head, 7¼; depth 14; snout 2¼; D.34 on 4+4 rings; its height 2 in base, which equals head. Rings 16+32. Nape slightly carinated. Color in alcohol, alternately annulated with light olive brown and dirty white; the dark colors on joints, the white on the bodies of the rings; dark color wider than white on trunk, narrower on caudal portion; white annulations on trunk between lateral and latero-ventral keels indicated by two narrow white lines with narrow black lines on either side and between, these portions of the whitish rings showing as silver bars in life and fresh alcoholic specimens; upper part of opercles dusky; a dark bar extending from the anterior edge of eye to end of snout; ventral keel, throat, lower part of opercles and snout, plain, whitish; dorsal with dark wavy diagonal bars. Other specimens vary in color from somewhat lighter to considerably darker than the above, the darker ones having some white mottling on throat, opercles, and beneath snout. Other females differ in much less depth, lower dorsal fin and in the color which ranges from almost plain olive through forms with reddish mottled appearance to brownish; fewer light-colored annulations and no distinct white or silver bars on sides.
>
> Description of male—Head 7½; depth 22½; snout 2¼; D. 33, on 4+4 rings; its height 2¾ in its base, which equals head. The male differs from the typical female in much less depth, lower dorsal fin, and in the coloration, all of which characters are those of shallow females. There is in the male, as in female, considerable color variation, but there are never any distinct white or silvery marks on the sides. Of the 130 specimens, 114 are females and young, 16 being adult males. Some of these were called by us *Siphostoma fuscum*. in the "Fishes of Texas and the Rio Grande Basin."

Jordan and Evermann (1896) state that:

> *Siphostoma scovelli* (Evermann and Kendall) 1896 was named for Dr. Joseph T. Scovell of Terre Haute, Indiana; that these specimens reached a length of 4½ inches and were common at Corpus Christi and perhaps elsewhere on the Gulf of Mexico. Apparently most of the published references to *S. affine* from the Gulf of Mexico belong to this species, which

PLATE III

Fig. 1 LOUISIANA AND A PORTION OF THE COAST OF MISSISSIPPI

differs from *S. affine* chiefly in having fewer body rings, in the more posterior position of the dorsal fin and in the fewer dorsal rays.

The aforementioned authors (1896) give the following historic review:

> The genus *Syngnathus* of Linnaeus, originally equivalent to the modern family of Syngnathidae was first subdivided by Rafinesque in 1810. The name *Siphostoma* was given to *S. pelagicus* and its relatives, the *Syngnathus* of late writers, that of *Tiphle* to *S. typhle,* the *Siphostoma* of late writers, while *Syngnathus* was retained for *S. aequoreus* and its relatives, the group now usually called *Nerophis,* the type of *Nerophis* being *Syngnathus ophidion,* L. This arrangement has been adopted here, but it is open to two objections besides the fact that it is contrary to the general usage, which makes *acus* the type of *Syngnathus,* in accordance with Swainson's arrangement. These objections are (1) that Artedi, from whom Linnaeus accepted the genus *Syngnathus,* did not know of the existence of *Syngnathus aequoreus,* and (2) the statement of Linnaeus (which we have been unable to verify), that the type of each of his genera is the "best known European or official species." *Syngnathus acus* would meet this requirement, but not *Syngnathus aequoreus,* which had not then been found in Europe. Should these objections be found valid, *Syngnathus* would take the place of *Siphostoma,* and *Nerophis* that of *Syngnathus.*

Herald (1941) reviewed the *Syngnathus californiensis* Storer complex. This paper is remarkable because of the author's discussion of sexual dimorphism, brood pouch appearance, egg laying and breeding season. Herald found that color had no taxonomic value in pipefish identification and concluded that some of the sub-speciation in the *S. californiensis* complex was of doubtful value.

Herald (1942) published a key to the Atlantic American species of pipefishes including *S. scovelli.*

Gunter (1942) noted that *S. scovelli* was known to inhabit most of peninsular Florida.

Gunter (1945) found *S. scovelli* with eggs in their brood pouches in June, August and November and a male with young in the brood pouch in October off the Texas coast. These ranged from 80-96 mm in length.

Breder (1948) incorrectly attributes *S. scovelli* to Evermann and Marsh 1902. His bibliographical reference to the Bulletin of the United States Fish Commission, Volume XX for 1900, published by the Government Printing Office in 1902 is in error and this work authored by Evermann and Marsh does not even contain a synonym for *S. scovelli.*

Reid (1954) reported conspicuous sexual dimorphism in *S. scovelli,* adult females marked with 15 to 18 vertical bars of silver on the trunk and considerably larger than males. Reid also made some observations on the size range of breeding males.

Gunter (1956) reported four marine syngnathids on the coasts of the western hemisphere which are known to be euryhaline *(Pseudophallus starksi, Syngnathus elcapitanensis, S. fuscus* and *S. scovelli.)* He also noted that Hildebrand reported a breeding population of *Oostethus lineatus* in fresh water; however, *O. lineatus* is not known to exist in pure sea water.

Eddy (1957) states that *S. scovelli* is very long and slender with a prehensile tail by which it clings to vegetation. This statement is incorrect for *S. scovelli* does not have a prehensile tail.

Simmons (1957), while studying the ecology of a hypersaline bay area, found that as salinity increased, the number of species decreased but the number of individuals of each species became greater. Simmons indicated that *S. scovelli* was common in salinities up to 45 o/oo.

Renfro (1960) reported a salinity range of 0.06-38.1 o/oo for *S. scovelli.*

Springer and Woodburn (1960) indicated a relationship between frequency of breeding and size in male *S. scovelli* and noted that breeding of *S. scovelli* took place all year with little change in incidence.

Herald (1961) relates some interesting facts about the mating behavior of *Syngnathus floridae,* the dusky pipefish found in the Gulf of Mexico but does not mention *S. scovelli.*

Herald (1961, personal communication) wrote:

> Although *S. scovelli* survives easily in salt water, no one as yet has kept the species alive in fresh water for more than a few days or at the most, a few weeks. I might mention that some of the best aquarists in the country have stubbed their toes on this one.

Prior to Whatley (1962) there is no mention of a breeding population of *S. scovelli* in fresh water.

Taylor (1966, personal communication) stated that the International Commission on Zoological Nomenclature, Opinion 45 (Smithsonian Institute Publication 2060, p. 101, 1912) tentatively designated *Syngnathus acus* Linnaeus as the type species of *Syngnathus.* In opinion 77 (Smithsonian Institute Publication 2657, p. 37, 1922) *S. acus* Linnaeus was fixed as the type species of *Syngnathus. S. scovelli* has been the correct name since 1912 as confirmed by the Commission in 1922. These opinions validate the objections stated by Jordan and Evermann (1896).

MATERIALS AND METHODS

A. Collecting

Among the numerous devices employed in attempts to capture *S. scovelli* during the period 23 July 1960 to 25 March 1966, were small meshed rectangular dip nets, a variety of minnow seines of varying mesh size, depth and lengths; several rectangular box-like collecting devices of light metal rods covered with alternate layers of fine aluminum screen and hardware cloth; and two types of electrical

shocking devices called "electric seines." These latter have been dubbed "the widow makers" by the Louisiana Wild Life and Fisheries Commission crews which use them. This equipment is described in detail by Witt and Campbell (1959). Marine pipefish were taken in seines, small rectangular dip nets, and by trawling. In the grass off Horn Island, the most successful device employed was the rectangular dip net.

Louisiana Wild Life and Fisheries Commission crews made several one-acre rotenone samples for me. The most recent of these, made 21 September 1965, provided 11 *S. scovelli.* This sample was made while I was present and proved the only effective rotenone sample made in Lake St. John as far as the collection of pipefish was concerned.

The most effective collecting device used to collect *S. scovelli* in Lake St. John proved to be a make-shift device pressed into service on 26 July 1960. This was an aquarium cover for a 50 gallon aquarium in the form of a rectangular wooden-framed shallow box 50 inches by 2 inches wide on the sides and 26 inches by 2 inches at the ends with two 26-inch braces spaced equidistant from the ends of the bottom to divide the bottom into three sectors. All wooden pieces were one-quarter inch fir. The bottom was of copper screening covered by one-quarter inch hardware cloth. Two handles (42 inch x 2 inch x 2 inch), which could be readily removed for convenience in transport, were fastened to the sides of this collecting device by long bolts. Two people, wading in water around waist deep, would slide this device along the lake bottom at a fairly sharp angle. When the device became filled with vegetation it was quickly raised to the level of the water surface and fishes trapped in the vegetation were removed by aquarium type nets which the persons operating the device wore around their necks on long cords. These fishes were then placed in plastic bags or buckets for transport. A child's wading ring supporting a plastic bucket full of lake water was tied by a long cord to the waist of one operator of the collecting device and provided a ready container in which pipefish could be kept with a minimum amount of handling. A supply of plastic bags which would hold five gallons of lake water was also carried.

B. Aquarium Studies

Numerous containers were used in attempts to keep *Syngnathus scovelli* alive for study purposes. These ranged from 50-gallon, 26-gallon, 10-gallon and 5-gallon glass aquaria to 25-gallon, 15-gallon, 10-gallon, 5-gallon, and 3-gallon plastic containers. Containers in which the fish were transported had been well washed before they were taken to the lake and were rinsed again in lake water before being used. Five-gallon jugs were filled with lake water each trip. This water was used to replace the water lost by evaporation and sloshing of water from the containers during transport.

Several types of aquatic organisms have been tried as food for *S. scovelli.* Brine shrimp *(Artemia salina)* eggs were hatched and the young brine shrimp fed to the fish. These were not only difficult to hatch in sufficient quantity but were expensive as well. A 5-pound container of eggs purchased for the purpose of feeding these fish had

a very low hatching percentage despite trials of a variety of methods for increasing hatching percentage. Mosquito larvae of small size were readily taken by the fish.

A plankton net was used to secure food for *S. scovelli* in the same location where the fish were taken. Plankton was also taken from Bayou DeSiard at Monroe, Louisiana by using the plankton net and a plankton light trap. Dr. A. J. Speece, formerly of Northeast Louisiana State College, now employed by Texas State Women's University of Denton, Texas, constructed a plankton light trap by using several pencil flashlight bulbs attached in series to a car battery. The bulbs, their magnifying tip having been dipped in an indelible blue ink and then allowed to dry, were suspended in a large test tube. This tube was surrounded by a cone of one-quarter inch mesh hardware cloth. A fine meshed cloth bag with a draw string was attached to the bottom of the cone of hardware cloth. Plankton attracted to the yellow light given off by the sides of the bulbs swam into the zone around the bulbs, were repelled by the blue light at the bottom of the bulbs and swam down into the bag. A 24-inch square piece of wood, one-inch thick, formed the float for each trap and the test tube was suspended through a three-inch wide opening bored in the center of the float.

RESULTS

A total of 44 collecting trips were made to Lake St. John from 23 July 1960 until 20 March 1966 resulting in the taking and preservation of 1,137 *S. scovelli* (Plate IV, Graph 2, and Table I).

During this same period other *S. scovelli* were maintained in various types of aquaria. One male taken 29 September 1960 survived until 16 November 1962 having lived in a 50-gallon aquarium with a filter of silk cloth under the sand for almost 27 months. Five other specimens taken 12 October 1960 survived until 18 March 1962, a period of 18 months, in a 20-gallon aquarium with an under-the-sand filter of silk. Numerous individual specimens survived from three to seven months each in fresh water containers of various types during this study.

A male taken 13 September 1960 gave birth to 16 offspring in a 5-gallon fresh water aquarium (Whatley 1962). Males with eggs in their brood pouches have been taken from March through October. Collecting trips during the other four months of the year have not produced any pipefish; therefore, the lack of male specimens with eggs in their brood pouches during these months does not necessarily reflect their absence in Lake St. John at those times. None of the young born in fresh water aquaria survived longer than five days.

S. scovelli ranging from 28 to 70 mm in total length were maintained in aquaria for several weeks. These specimens grew at a rapid rate until their length approached 80 mm at which time the growth rate slowed sharply. Indications of three year classes of individuals (0: 12-80-mm, 1: 80-120 mm and 2: 120-160 mm) were present, but consecutive monthly data were not enough to prove this point.

Many of the *S. scovelli* taken from Lake St. John have been found enmeshed in naiad *(Najas guadalupensis)*. When naiad was placed in aquaria with the pipefish, they maintained themselves in it in an almost horizontal position with the tip of the snout being slightly elevated. In this vegetation the pipefish appeared slightly green in color, but they did not approach the vivid colors present at the time they were taken from Lake St. John. Specimens freshly taken from the lake vary greatly in color but their colors are generally bright. Some forms have rich brown backs with darker brown to black rings, intergrading through lighter brown sides with an iridescent golden or metallic green sheen. The belly on some was a creamy white with yellow lateral margins, while others were uniformly light, yellowish brown or grey on the back with very light grey bellies. Evermann and Kendall (1896) described the preserved forms very well with regard to coloration, a description which has been given in the literature review.

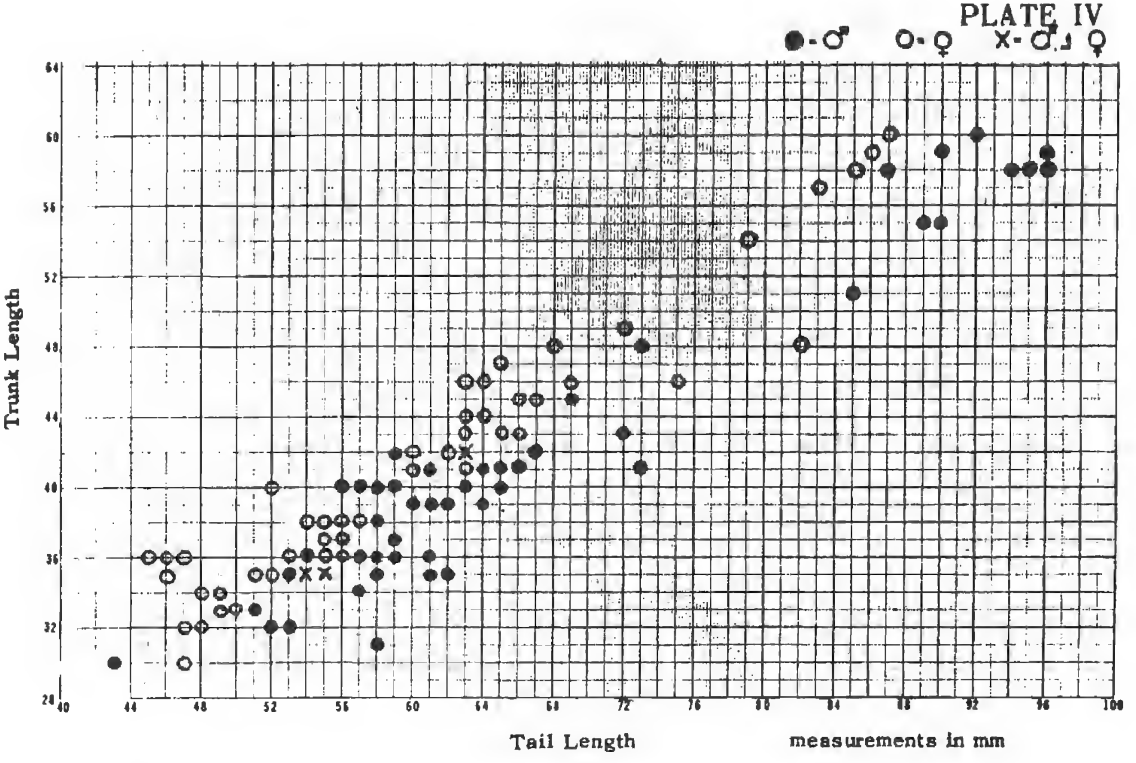
I took *S. scovelli* around several aquatic plants other than the naiad previously listed. Some of these are: (1) water-millet *(Zizaniopsis miliacea)*, which forms a border around most of Lake St. John; (2) bald cypress *(Taxodium distichum)*, which grow at the shore-line, about 50-yards out from shore and about 100 yards out from much of the east shore of the lake, along most of the rest of the shore and in the shallower water area at the extreme northwestern portion of the lake; (3) American lotus *(Nelumbo pentapetala)* which is found in patches 20 to 30 yards out from the east and west shores.

Other aquatics which proved disappointing as pipefish habitat were: (1) coontail *(Ceratophyllum demersum)*, (2) alligator weed *(Altermanthera philoxeroides)*, (3) duckweed *(Lemna minor)*, and (4) willow primrose *(Jussiaea grandiflora* and *J. diffusa)*. The classification of aquatics listed above is according to Muenscher (1944) except for *Z. miliacea* which is according to Fernald (1950).

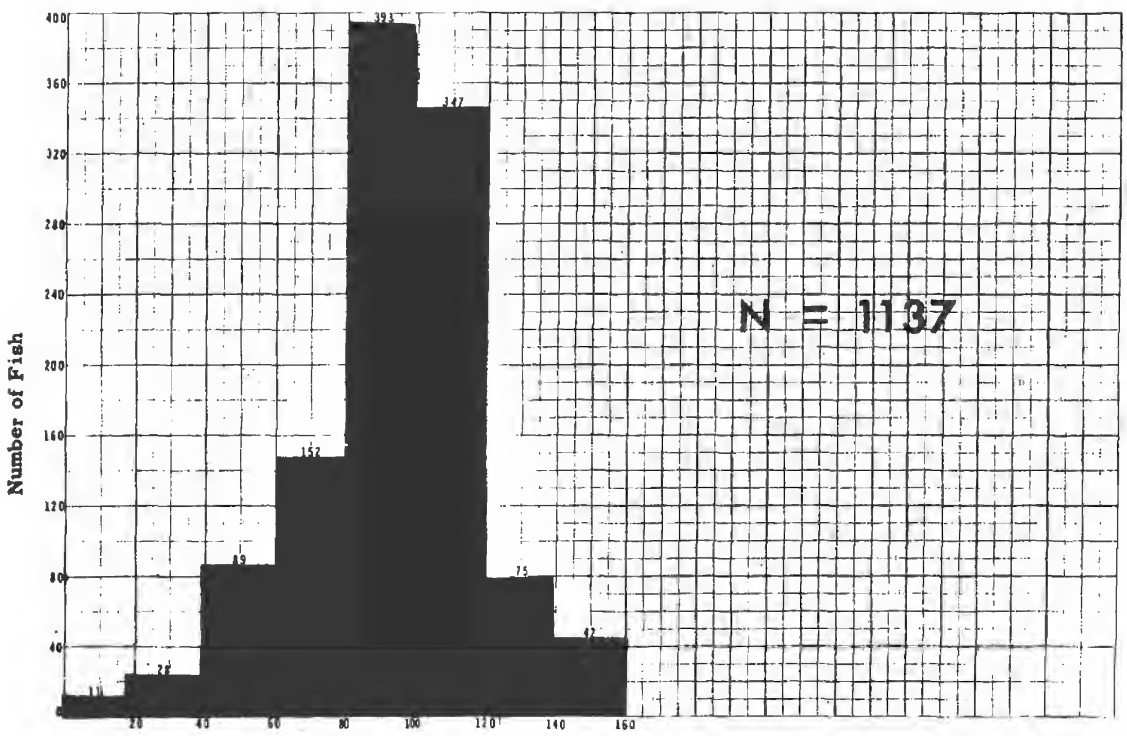
Plankton was plentiful in Lake St. John during the first part of this study. Phytoplankton, rotifers, copepods and ostracods were abundant in plankton samples taken along with pipefish. Plankton organisms in plastic bags of lake water were placed on ice in insulated containers but did not remain alive for a very long period of time. It was necessary to collect plankton nightly at Bayou DeSiard to supplement the plankton taken in the light traps previously mentioned. These fish seem to have an extremly high metabolic rate. They did not stop feeding as long as plankton remained in the tank. Pipefish are tireless hunters and the independent action of their eyes is quite helpful in feeding. Plankton organisms on either side at different levels are spotted quite readily and taken in with quick movements of the snout, up and down, right and left.

Many of the fishes taken in the collecting devices along with pipefish have been found competing with the pipefish for plankton. This is especially true of the young of the year of the various sunfishes, darters, silversides, madtoms, mosquito fish, shad, and the various killifishes in Lake St. John. The gars, bowfin, bass, white crappie, black crappie, and the larger sunfishes are predatory and feed on the plankton feeders. The grass shrimp *(Palaemonetes kadiakensis)* also actively competes for plankton in Lake St. John. This same relation

PLATE IV



GRAPH NO. 1 Scatter graph plotting trunk length against tail length of 50 female and 50 male specimens of Syngnathus scovelli from Lake St. John, Louisiana.



GRAPH NO. 2 Histogram of length frequencies of 1137 specimens of Syngnathus scovelli collected in Lake St. John, Louisiana from July 1960 through March 1966.

exists in Davis Bayou and off Horn Island with other species of *Palaemonetes*. Young of the year of many fishes are plankton feeders. These form the base of the food chain and predatory fishes come into the shallows and feed on the plankton feeders.

On 7 August 1961 seven *S. scovelli* in a 10-gallon aquarium were given plankton which had not been strained. This plankton contained *Dugesia tigrina*. Almost immediately a number of these flatworms attached themselves to the gills of three living pipefish whereupon the pipefish began writhing about on the bottom of the tank. It was soon apparent that the pipefish could not dislodge the flatworms, and they were removed from the tank so that the flatworms could be picked off with forceps. The four unharmed pipefish were removed from the tank and suffered no ill effects. Those three specimens to which the flatworms had attached did not recover.

S. scovelli were fairly abundant around the natural springs welling up into the Lake St. John shallows off the east shore near Blackwood Landing in July and August of 1960. The water temperature around these springs averaged 10 F cooler than the surrounding bottom waters and about 20 F cooler than the surface waters in direct sunlight. Optimum temperature for *S. scovelli* appears to range between 72 F and 80 F. Activity greatly slowed down in tanks when temperature of the water rose to 82 F. Very few specimens survived the trip from Lake St. John to Monroe, Louisiana (a distance of around 100 miles) when the water temperature in the fish containers approached 85 F. Plastic bags containing lake water and fish were placed in ice chests in which a small amount of crushed ice had been placed. This lowered the temperature to between 70 F and 80 F and proved to be a successful technique in transporting the fish. The most successful trips with living fish were those made early in the morning or late at night when the daily air temperature was at its lowest point.

Movement of *S. scovelli* from place to place in aquaria is accomplished by joint movement of the pectoral fins and the dorsal fin. In the dorsal fin S-shaped waves of movement progressing along its length are evident when the fish is viewed from above. The fish is not a rapid swimmer but its movements from side to side are deceptively quick.

Most of the 1,137 specimens of *S. scovelli* collected and preserved (Plate IV, Graph 2) were taken prior to 12 October 1961. At that time a Louisiana Wild Life and Fisheries Commission crew under the direction of Mr. Tommy Allen, then employed in the Ferriday, Louisiana office of the Louisiana Wild Life and Fisheries Commission, placed a great deal of rotenone in the lake in an attempt to effect a partial shad *(Dorosoma cepedianum* and *Dorosoma petenense)* kill. This effort did not appear to be very successful as far as shad were concerned, but it seemed to be extremely effective on pipefish and naiad. On 17 October 1961 I waded through a fishy, smelly, decaying mass of organic matter in search of pipefish. No living pipefish were taken at this time nor for some time thereafter. On 22 June 1962, 27 *S. scovelli* were taken, 11 of these being only 18 mm in length. The population is slowly returning. The naiad *(Najas guadalupensis)* has not been plentiful since 12 October 1961. Only scattered sprigs seem to exist over most of the lake. The most recent additions to the collection

TABLE I

Summary of data from collecting trips made to Lake St. John, Louisiana from 23 July 1960 through 20 March 1966.

	Date	Range In Size Of Specimens In Millimeters	Total Number Of Specimens Collected and Preserved On Each Date
1.	23 July 1960	60 -	1
2.	26 July 1960	80 - 155	26
3.	30 July1960	65 - 145	12
4.	11 August 1960	65 - 142	45
5.	14 August 1960	72 - 105	69
6.	26 August 1960	68 - 147	203
7.	13 September 1960	80 - 108	52
8.	15 September 1960	82 - 105	44
9.	29 September 1960	68 - 127	18
10.	12 October 1960	71 - 112	208
11.	18 December 1960	-	none
12.	18 June 1961	65 - 152	12
13.	18 July 1961	24 - 122	92
14.	14 August 1961	77 - 112	74
15.	7 October 1961	44 - 160	77
16.	10 October 1961	60 - 127	127
17.	17 October 1961		none
18.	17 December 1961		none
19.	19 April 1962		none
20.	2 May 1962		none
21.	16 May 1962		none
22.	22 June 1962	18 - 128	27
23.	27 August 1962	47 - 137	14
24.	17 September 1962		none
25.	19 September 1962		none
26.	22 January 1963		none
27.	15 February 1963		none
28.	14 March 1963		none
29.	27 April 1963		none
30.	30 May 1963	72 - 118	6
31.	13 August 1963	86 - 115	11
32.	15 September 1963		none
33.	2 September 1964		none
34.	9 September 1964		none
35.	21 September 1965	89 - 119	11
36.	28 September 1965		none
37.	12 October 1965		none
38.	19 October 1965		none
39.	17 December 1965		none
40.	29 January 1966		none
41.	12 February 1966		none
42.	19 February 1966		none
43.	18 March 1966	98 - 142	4
44.	20 March 1966	110 - 157	4
		Total	1,137

are four specimens taken on 20 March 1966. Approximately 356 hurs were spent collecting at Lake St. John. Much of this collecting effort was unsuccessful after rotenone was placed in the lake 12 October 1961.

During the period July 1960 to March 1966, 27 collecting trips were made to other oxbow lakes of the Mississippi River relatively near Lake St. John. Allocation of these trips was as follows: Lake Bruin was visited 19 times; Lake Concordia, 6 times, and Lake St. Joseph, 2 times. I did not find any pipefish in any of these other lakes; however, nine *S. scovelli* have been given to me by other persons who collected them in Lake Bruin. This is the only lake near Lake St. John known to contain pipefish at this time. Approximately 162 hours were spent in collecting attempts in lakes other than Lake St. John.

Mr. C. E. Dawson of the Gulf Coast Research Laboratory examined several of 27 specimens of pipefish taken to the Gulf Coast Research Laboratory in August 1960 and identified the specimens as representative of the population *S. scovelli*. I have seen more than 1,200 specimens from Lake St. John since the first one was found on 23 July 1960 and my collection has recently been carefully checked by Dr. Neil Douglas of Northeast Louisiana State College. A check of 500 specimens preserved reveals a dorsal fin ray count of 35, a pectoral fin ray count of 16, a caudal fin ray count of 10 and an anal fin ray count of five. This population appears to be extremely homogenous throughout. Table I does not reveal any monthly variation in size which is striking. Apparently this population breeds practically every month in the year and failure to collect a greater size range of individuals each time could very well be due to sampling methods employed and to poisoning of the lake.

A. Observations on *S. scovelli* from Mississippi Coastal Waters

In the summer of 1964 and 1965 I collected *S. scovelli* specimens from Davis Bayou near Ocean Springs, Mississippi, and off Horn Island which is off the coast of Mississippi (Plate III, Figs. 1, 3 and 4). These were compared with *S. scovelli* from Lake St. John and the nine specimens from Lake Bruin. No differences could be detected which would indicate that those fish in Lake St. John should be placed in a different species.

S. scovelli were maintained alive in 3-gallon plastic buckets at the Gulf Coast Research Laboratory in June, July, and August of 1964 and in July and August of 1965. In 1964 a number of these fish were transported to Monroe, Louisiana, and some of these were kept alive until March of 1965.

On three occasions, twice in August of 1964 and once in July of 1965, cannibalism was observed in male *S. scovelli* which had given birth to offspring and were eating them almost as rapidly as they were born. In all three cases the males were alone in 3-gallon plastic buckets. Two of these males were preserved after they had eaten around 16 young pipefish each.

On 6 August 1964, a male *S. scovelli* from the vicinity of Horn

TABLE II

Species of fishes other than *Syngnathus scovelli* taken in Lake St. John from 23 July 1960 through 25 March 1966.

Family	Occurrence
1. Lepisosteidae	
Lepisosteus oculatus	common
Lepisosteus osseus	common
Lepisosteus platostomus	common
Lepisosteus spatula	rare
2. Amiidae	
Amia calva	common
3. Clupeidae	
Dorosoma cepedianum	common
Dorosoma petenense	common
4. Cyprinidae	
Cyprinus carpio	common
Notemigonus crysoleucas	common
Notropis maculatus	common
Opsopoeodus emiliae	common
Pimephales vigilax	common
5. Catostomidae	
Erimyzon sucetta	rare
Ictiobus cyprinellus	rare
Ictiobus niger	rare
6. Ictaluridae	
Ictalurus melas	common
Ictalurus natalis	common
Ictalurus punctatus	rare
Noturus gyrinus	common
7. Cyprinodontidae	
Fundulus chrysotus	common
Fundulus notatus	common
Fundulus notti	common
Fundulus olivaceus	common
8. Poecilidae	
Gambusia affinis	common
9. Aphredoderidae	
Aphredoderus sayanus	rare
10. Serranidae	
Roccus mississippiensis	rare
11. Centrarchidae	
Centrarchus macropterus	rare
Chaenobryttus gulosus	common
Elassoma zonatum	rare
Lepomis cyanellus	common
Lepomis humilis	rare
Lepomis macrochirus	common
Lepomis microlophus	common
Lepomis punctatus	common
Lepomis symmetricus	rare
Micropterus salmoides	common
Pomoxis annularis	common
Pomoxis nigromaculatus	common
12. Percidae	
Etheostoma barratti	rare
Etheostoma chlorosomum	rare
Etheostoma proeliare	rare
13. Scianeidae	
Aplodinotus grunniens	rare
14. Atherinidae	
Labidesthes sicculus	common
Menidia audens	rare

Island was placed in a 3-gallon plastic bucket with a female *S. scovelli* from the mouth of Davis Bayou. This male had given birth to 16 offspring on 5 August 1964, and these offspring had been left in a bucket to themselves. At 1815 on the evening of 6 August 1964 the female was observed coming to the surface of the bucket and violently shaking her head from side to side. After about 20 minutes the male swam to the surface and joined the female. Their bodies were erect in the water, forming S-shaped curves. There was a much closer resemblance to the sea horse body form than I would have believed possible. Dorsal fins of both fish were erect and flaring. After swimming around each other and briefly twining bodies for some 10 to 15 seconds, the male approached the female from the rear, (her ovipositor was apparent all of the time), placed his tail around hers and the pair spun around together in the center of the bucket for a very brief interval, approximately 3 to 5 seconds. During this period the female's ovipositor was placed in the brood pouch of the male. This behavior was repeated a second time at 1830. After this the pair was observed until 1930 and no further mating behavior occurred. The male was taken from the container and it was found that his brood pouch was filled with eggs. This indicates either that oviposition is very rapid or that only the end of the mating period was observed. The male was placed in a separate container, and on 18 August 1964 produced 32 young pipefish. He was placed in the container with the same female with which he had previously mated on the night of 18 August 1964. At 0600, 19 August 1964, his brood pouch was found to be filled with eggs again. The young were born on 30 August 1964. Incubation of the eggs in the brood pouch is apparently dependent upon the temperature. Some pipefish collected with eggs in their brood pouches have taken as long as 16 days to have their young.

Young pipefish were given plankton taken by plankton net from the boat slip at Gulf Coast Research Laboratory. This plankton was strained through cheese cloth which had been folded four times. Their rate of growth was extremely rapid, especially the initial growth. From 27 June 1964 until 7 July 1964, their increase in length was from 12 mm at birth to 28 mm in less than two weeks time. As in the fresh water specimens, the rate of growth seems extremely rapid at first. From 27 June 1964 until 18 September 1964, three specimens grew from 12 mm to 60 mm. These three specimens died on 29 September 1964 and were preserved at that time. This represents a 5-fold increase in length in a period of a little more than three months.

S. scovelli, whether they are taken in a fresh water lake (Lake St. John), a brackish water environment (Davis Bayou), or a marine habitat (off Horn Island) appear to be gregarious animals until the brood pouch of the male is filled with eggs. In all three of the locations mentioned, males with eggs were not uncommonly taken by themselves in seine hauls. Pipefish placed in aquaria assemble in one end of the aquarium. On several occasions males with eggs in the brood pouch moved away from the group to the other end of the tank. These were removed and isolated in tanks where the males with eggs assembled in groups. This gregarious behavior apparently is not controlled entirely by feeding. Even when no apparent food

TABLE III

Species of fishes other than *Syngnathus scovelli* collected from Davis Bayou near the Gulf Coast Research Laboratory at Ocean Springs, Mississippi during the summer of 1964 and the Summer of 1965.

Family	Occurrence
1. Dasyatidae	
Dasyatis sabina	rare
2. Lepisosteidae	
Lepisosteus oculatus	common
Lepisosteus platostomus	common
3. Clupeidae	
Brevoortia patronus	common
Dorosoma cepedianum	rare
Dorosoma petenense	rare
4. Engraulidae	
Anchoa hepsetus	common
Anchoa mitchilli	rare
5. Synodontidae	
Synodus foetens	common
6. Ariidae	
Galeichthys felis	common
7. Anguillidae	
Anguilla rostrata	rare
8. Ophichthidae	
Ahlia egmontis	common
Myrophis punctatus	common
9. Cyprinodontidae	
Adinia xenica	rare
Cyprinodon variegatus	common
Fundulus grandis	common
Fundulus jenkinsi	rare
Fundulus similis	common
Lucania parva	rare
10. Poeciliidae	
Gambusia affinis	common
Mollienesia latipinna	common
11. Syngnathidae	
Syngnathus louisianae	rare
12. Centrarchidae	
Lepomis macrochirus	common
Lepomis cyanellus	rare
Micropterus salmoides	common
13. Carangidae	
Chloroscombrus chrysurus	rare
Oligoplites saurus	common
14. Pomadasyidae	
Orthopristis chrysopterus	common
15. Sciaenidae	
Bairdiella chrysurus	common
Cynoscion arenarius	common
Cynoscion nebulosus	rare
Leiostomus xanthurus	common
Menticirrhus americanus	rare
Menticirrhus focaliger	common
Micropogon undulatus	common
Sciaenops ocellata	common
16. Sparidae	
Lagodon rhomboides	common
17. Gobiidae	
Gobionellus schufeldti	rare
18. Blenniidae	
Chasmodes saburrae	rare
Hypsoblennius ionthas	rare
19. Mugilidae	
Mugil cephalus	common
Mugil curema	common
20. Atherinidae	
Membras martinica vagrans	common
Menidia beryllina	common
21. Bothidae	
Citharichthys spilopterus	common
Etropus crossotus	rare
Paralichthys albigutta	rare
Paralichthys lethostigma	common
22. Soleidae	
Trinectes maculatus	common
23. Cynoglossidae	
Symphurus plagiusa	common
24. Batrachoididae	
Opsanus beta	common

is present in aquaria, and no apparent feeding activity is evident, pipefish appear to remain in groups. The salinity range reported for *S. scovelli* is a wide one. Renfro (1960) gives a range of 0.06-38.1 o/oo for the species while Simmons (1957) indicated that *S. scovelli* was common in salinities up to 45 o/oo.

B. Associated Fishes in Fresh Water

Lake St. John collections produced 45 species of fishes other than *S. scovelli* representing 14 families (Table II). Lambou (1961) lists 29 species of fishes from oxbow lakes of the Mississippi River (these include Lake St. John); however, Lambou's listing—mostly minnows *(Cyprinidae)* and other small fishes included most of the other species listed in this paper. Lambou lists *Strongylura marina* as one of his 29 species but does not give the lake in which it was taken or its length. Dr. William R. Taylor, associate curator, Division of Fishes, Smithsonian Institution, United States National Museum, by means of a personal communication has given me permission to use his field notes made while employed in the Monroe, Louisiana, office of the Louisiana Wild Life and Fisheries Commission. These notes reveal that the specimen *S. marina* listed above was taken at False River (one of the oxbow lakes in Lambou's paper) on 25 May 1955 and that this fish was an adult specimen 485 mm in length.

C. Associated Fishes in the Salt Waters of Mississippi

Davis Bayou yielded 51 species of fishes other than *S. scovelli* representing 24 families (Table III). Davis Bayou is a brackish water environment.

Horn Island inlets, ponds and the waters offshore on the Mississippi Sound side are represented by 102 species of fishes from 47 different families (Table IV). Richmond (1962) lists 61 fishes from 32 families from the Horn Island area. My notations, rare or common, in the three tables listed above relate only to my collection of specimens and are not intended to reflect general scarcity or abundance.

The number of species of fishes and the number of families in Table II and Table IV appear to represent a fair sampling of the fish fauna of Lake St. John and the Horn Island area when compared with the publications of Lambou (1961) and Richmond (1962).

D. Sexual Dimorphism

Fully mature female *S. scovelli* have comparatively longer trunks and shorter tails than fully mature male *S. scovelli.* This observation led to an attempt to correlate these factors with regard to the sexes. Fifty females and 50 males were selected from the population of 1,137 preserved specimens. Millimeter measurements were used. A ratio was established by dividing trunk length into tail length and the results were plotted on a scatter diagram graph (Plate IV, Graph 1). The results of the analysis of these data are given in Table V. These data were significant at the 95% level. The selection of specimens was necessary to establish 50 different measurements of each sex. This selection should be taken into consideration when evaluating the data. Mature females showed considerable variation in the ratio of

trunk to tail length. A variation of 1.45 in females to 1.62 in males at the high extreme of body length in millimeters was shown.

Herald (1942) gives the following criteria for counts and measurements on pipefishes: (1) all fin rays are counted, (2) the first trunk ring is the ring bearing the pectoral fins, and the last is that bearing the anus, (3) the hypural ring is not included in the tail ring count, (4) the head measurement is taken from the most anterior part of the fish, with the mouth firmly closed, to the posterior end of the opercular bone (care must be exercised in determining this latter point, and often microscopic examination is required), and (5) the snout is measured from the tip to the posterior end of the preorbital bone.

With the above points in mind, some final points concerning the population of *S. scovelli* (Plate I) in Lake St. John are listed: (1) snout-in-head 2.0 to 2.55, (2) trunk segments 17, (3) caudal segments 30-32, (4) dorsal fin rays 35, (5) dorsal fin covering 3 trunk segments and 5 tail segments, (6) brood pouch covering 11-13 tail segments (generally 12), (7) pectoral rays 16, (8) anal rays 5, (9) caudal rays 10. Adult male and female specimens are readily distinguishable from each other. The "V" bellied females have comparatively deeper trunks and shorter tails. The males are more flat bellied, have more slender trunks and longer tails (Plate I, Figs. 1 and 2, Plate II, Figs. 1, 2, 3, 4, 5 and 6). Forms ranging from 80 mm to 115 mm are frequently hard to distinguish with regard to sex and not infrequently dissection of the pipefish in question is required.

The original description of Evermann and Kendall in 1896 listed 16 trunk segments for *Siphostoma scovelli*. Herald's criteria are responsible for the discrepancy in number of trunk segments, not a variation in the animals. The marine and brackish water *S. scovelli* examined constitute a more heterogenous population with regard to meristic characters than the fresh water population in that dorsal fin ray counts vary from 29-35, and pectoral fin rays range from 14 to 16 in the marine species. A statistical analysis of the dorsal fin ray counts of 81 marine *S. scovelli* from the vicinity of Horn Island, Mississippi, compared with the dorsal fin ray counts of 500 fresh water *S. scovelli* from Lake St. John, Louisiana, is given in Table VI.

The eggs in the brood pouch of *S. scovelli* are arranged in two rows and va[illegible] 16 to 64. Common litter size noted was 16. This may be [illegible]balism and more fish may be born but a number ma[illegible]

SUMMARY AND CONCLUSIONS

More than 1,200 specimens of *S. scovelli* have been taken from Lake St. John in inland Louisiana. This population is strikingly homogeneous with regard to ring and fin ray counts and probably has not existed in Lake St. John for much over 1,000 years. Herre (1927), working with gobies in the Philippine Islands and the China Sea, found that isolation of these fishes for 10,000 years produced 77 genera and 173 species. The small variation in the *S. scovelli* population in Lake St. John would seem to indicate the establishment of a fairly

TABLE IV

Species of fishes other than *Syngnathus scovelli* taken in ponds and inlets on Horn Island and from offshore on the Mississippi Sound side off Horn Island during the Summer of 1964 and the Summer of 1965.

Family	Occurrence
1. Caracharhinidae	
Scoliodon terraenovae	rare
2. Torpedinidae	
Narcine brasiliensis	rare
3. Dasyatidae	
Dasyatis americana	rare
Dasyatis sabina	common
Dasyatis sayi	common
Gymnura micrura	rare
4. Elopidae	
Elops saurus	common
5. Clupeidae	
Brevoortia patronus	common
Harengula pensacolae	common
Opisthonema oglinum	rare
6. Engraulidae	
Anchoa hepsetus	common
Anchoa mitchilli diaphana	commo
7. Synodontidae	
Synodus foetens	common
8. Ariidae	
Bagre marina	rare
Galeichthys felis	common
9. Ophichthidae	
Ahlia egmontis	common
Myrophis punctatus	common
Ophichthus gomesi	rare
10. Belonidae	
Strongylura marina	common
11. Hemiramphidae	
Hyporhamphus unifasciatus	rare
12. Cyprinodontidae	
Adinia xenica	rare
Cyprinodon variegatus	common
Fundulus grandis	common
Fundulus similis	common
Lucania parva	rare
13. Poeciliidae	
Gambusia affinis	common
Mollienesia latipinna	common
14. Gadidae	
Urophycis floridanus	common
15. Syngnathidae	
Hippocampus erectus	rare
Hippocampus zosterae	common
Syngnathus floridae	rare
Syngnathus louisianae	common
16. Serranidae	
Centropristes philadelphicus	rare
17. Lobotidae	
Lobotes surinamensis	rare
18. Lutjanidae	
Lutjanus griseus	rare
19. Rachycentridae	
Rachycentron canadum	rare
20. Carangidae	
Caranx bartholomaei	rare
Caranx crysos	common
Caranx latus	rare
Chloroscombrus chrysurus	common
Oligoplites saurus	common
Selene vomer	rare
Trachinotus carolinus	common
Trachurus lathami	common
Vomer setapinnis	rare
21. Gerridae	
Eucinostomus argenteus	rare
Eucinostomus gula	rare
22. Pomadasyidae	
Orthopristis chrysopterus	common
23. Sciaenidae	
Bairdiella chrysura	rare
Cynoscion arenarius	common
Cynoscion nebulosus	common
Equetus acuminatus	rare
Larimus fasciatus	rare
Leiostomus xanthurus	common
Menticirrhus americanus	rare
Menticirrhus focaliger	common
Menticirrhus littoralis	common
Micropogon undulatus	common
Pogonias cromis	common
Sciaenops ocellata	common
Stellifer lanceolatus	rare

TABLE IV

(Continued)

Family	Occurrence
24. Sparidae	
Archosargus probatocephalus	common
Lagodon rhomboides	common
Stenotomus caprinus	rare
25. Ephippidae	
Chaetodipterus faber	common
26. Trichiuridae	
Trichiurus lepturus	common
27. Scombridae	
Scomberomorus cavalla	rare
Scomberomorus maculatus	common
28. Gobiidae	
Gobionellus boleosoma	rare
Gobionellus hastatus	common
29. Triglidae	
Prionotus evolans	rare
Prionotus rubio	common
Prionotus scitulus latifrons	rare
30. Uranoscopidae	
Astroscopus y-graecum	rare
31. Dactyloscopidae	
Dactyloscopus tridigitatus	rare
32. Blenniidae	
Chasmodes saburrae	rare
Hypsoblennius ionthas	rare
33. Ophidiidae	
Rissola marginata	rare
34. Stromateidae	
Peprilus paru	rare
Poronotus triacanthus	common
35. Mugilidae	
Mugil cephalus	common
Mugil curema	common
36. Atherinidae	
Membras martinica vagrans	common
Menidia beryllina	common
37. Polynemidae	
Polydactylus octonemus	common
38. Bothidae	
Ancylopsetta quadrocellata	rare
Citharichthys spilopterus	common
Etropus crossotus	rare
Paralichthys albigutta	common
Paralichthys lethostigma	common
39. Soleidae	
Trinectes maculatus	common
40. Cynoglossidae	
Symphurus plagiusa	common
41. Gobiesocidae	
Gobiesox strumosus	common
42. Balistidae	
Alutera schoepfi	rare
43. Ostraciidae	
Lactophrys quadricornis	common
44. Tetraodontidae	
Lagocephalus laevigatus	common
Sphaeroides nephelus	common
45. Diodontidae	
Chilomycterus schoepfi	common
46. Batrachoididae	
Opsanus beta	common
47. Antennariidae	
Antennarius radiosus	rare
Histrio histrio	rare

TABLE V

Statistical analysis of tail length to trunk ratio in female and male *Syngnathus scovelli.*

Female				Male			
FISH NO.	TRUNK LENGTH	TAIL LENGTH	RATIO	FISH NO.	TRUNK LENGTH	TAIL LENGTH	RATIO
1.	30	47	1.56	1.	30	43	1.43
2.	32	47	1.46	2.	33	58	1.75
3.	32	48	1.50	3.	32	52	1.62
4.	33	49	1.48	4.	32	53	1.65
5.	33	50	1.51	5.	33	51	1.53
6.	34	48	1.41	6.	34	57	1.67
7.	34	49	1.44	7.	35	53	1.51
8.	35	46	1.31	8.	35	54	1.54
9.	35	51	1.45	9.	35	55	1.57
10.	35	52	1.48	10.	35	58	1.65
11.	35	54	1.54	11.	35	61	1.74
12.	35	55	1.57	12.	35	62	1.77
13.	36	45	1.25	13.	36	54	1.50
14.	36	46	1.27	14.	36	57	1.58
15.	36	47	1.30	15.	36	58	1.61
16.	36	53	1.47	16.	36	59	1.63
17.	36	55	1.52	17.	36	61	1.69
18.	36	56	1.55	18.	37	59	1.59
19.	37	55	1.48	19.	38	58	1.52
20.	37	56	1.51	20.	39	60	1.53
21.	38	54	1.42	21.	39	61	1.56
22.	38	55	1.44	22.	39	62	1.58
23.	38	56	1.47	23.	39	64	1.64
24.	38	57	1.50	24.	40	56	1.40
25.	40	52	1.30	25.	40	57	1.42
26.	41	60	1.46	26.	40	58	1.45
27.	41	63	1.53	27.	40	59	1.47
28.	42	60	1.42	28.	40	63	1.57
29.	42	62	1.47	29.	40	65	1.62
30.	42	63	1.50	30.	41	61	1.48
31.	43	63	1.46	31.	41	64	1.56
32.	43	65	1.51	32.	41	65	1.58
33.	43	66	1.53	33.	41	66	1.60
34.	44	63	1.43	34.	41	73	1.78
35.	44	64	1.45	35.	42	59	1.40
36.	45	65	1.44	36.	42	63	1.50
37.	45	66	1.46	37.	42	67	1.59
38.	46	63	1.36	38.	43	72	1.67
39.	46	64	1.39	39.	45	69	1.53
40.	46	69	1.50	40.	48	73	1.52
41.	46	74	1.60	41.	51	85	1.66
42.	47	65	1.38	42.	55	89	1.61
43.	48	68	1.41	43.	55	90	1.63
44.	48	82	1.70	44.	57	87	1.52
45.	49	72	1.46	45.	58	94	1.62

TABLE V

(Continued)

Female				Male			
FISH NO.	TRUNK LENGTH	TAIL LENGTH	RATIO	FISH NO.	TRUNK LENGTH	TAIL LENGTH	RATIO
46.	54	79	1.46	46.	58	95	1.63
47.	57	83	1.45	47.	58	96	1.65
48.	58	85	1.46	48.	59	90	1.52
49.	59	86	1.45	49.	59	96	1.62
50.	60	87	1.45	50.	60	92	1.53

100 selected specimens—50 males plus 50 females
All specimens listed above taken from Lake St. John, Louisiana
All measurements are in millimeters

Females				Males			
X	X^2	X	X^2	X	X^2	X	X^2
1.56	2.4336	1.46	2.1316	1.43	2.0449	1.45	2.1025
1.46	2.1316	1.53	2.3409	1.75	3.0625	1.47	2.1609
1.50	2.2500	1.42	2.0164	1.62	2.6244	1.57	2.4649
1.48	2.1904	1.47	2.1609	1.65	2.7225	1.62	2.6244
1.51	2.2801	1.50	2.2500	1.54	2.3716	1.48	2.1904
1.41	1.9881	1.46	2.1316	1.67	2.7889	1.56	2.4336
1.44	2.0736	1.51	2.2801	1.51	2.2801	1.58	2.4964
1.31	1.7161	1.53	2.3409	1.54	2.3716	1.60	2.5600
1.45	2.0125	1.43	2.0449	1.57	2.4949	1.78	3.1684
1.48	2.1904	1.45	2.1025	1.65	2.7225	1.40	1.9600
1.54	2.3716	1.44	2.0736	1.74	3.0276	1.50	2.2500
1.57	2.4649	1.46	2.1316	1.77	3.1329	1.59	2.5281
1.25	1.5625	1.36	1.8496	1.50	2.2500	1.67	2.7889
1.27	1.6129	1.39	1.9321	1.58	2.4964	1.53	2.3409
1.30	1.6900	1.50	2.2500	1.61	2.5921	1.52	2.3104
1.47	2.1609	1.60	2.5600	1.63	2.6569	1.66	2.7556
1.52	2.3104	1.38	1.9044	1.69	2.8561	1.61	2.5921
1.55	2.4025	1.41	1.9881	1.59	2.5281	1.63	2.6569
1.48	2.1904	1.70	2.8900	1.52	2.3104	1.52	2.3104
1.51	2.2801	1.46	2.1316	1.53	2.3409	1.62	2.6244
1.42	2.0164	1.46	2.1316	1.56	2.4336	1.63	2.6569
1.44	2.0736	1.45	2.1025	1.58	2.4964	1.65	2.7225
1.47	2.1609	1.46	2.1316	1.64	2.6896	1.52	2.3104
1.50	2.2500	1.45	2.1025	1.40	1.9600	1.62	2.6234
1.30	1.6900	1.45	2.1025	1.42	2.0164	1.53	2.3409
	$EX = 72.92$			$EX = 79.00$			
	$EX^2 = 106.6750$			$EX^2 = 125.2156$			
	$E(X)^2/50 = 106.3465$			$E(X)^2/50 = 124.8200$			
	$\overline{X} = 1.4584$			$\overline{E} = 1.5800$			

TABLE V

(Continued)

$$S\frac{2}{x} = \frac{125.2156 - 124.8200 + 106.6750 - 106.3465}{50 + 50 - 2}$$

$$S\frac{2}{x} = \frac{0.7241}{98} = 0.074$$

$$S\frac{2}{x_1 - x_2} = .0074\,(1/50 + 1/50) = .0074 \times \frac{2}{50} = \frac{.0148}{50} = 0.000296$$

$$S_{\bar{x}_1 - \bar{x}_2} = \sqrt{1.000296} = 0.0172$$

$$T = \frac{1.5800 - 1.4584}{0.0172} = \frac{0.1216}{0.0172} = 7.06$$

T_{c} 98 df =

T_{c} 60 df = 2.000

T_{c} 120 df = 1.980

T of 7106 is greater than T_{c} 60 df 2.000 T_{c} 120 df 1.980. Therefore trunk length ratio of males over females is significant at the 95% level.

The procedure used here is that of Steele and Torrie (1960).

TABLE VI

Statistical analysis of fin ray count of 81 *Syngnathus scovelli* specimens from vicinity of Horn Island, Mississippi compared to fin ray counts of 500 *Syngnathus scovelli* from Lake St. John, Louisiana.

Dorsal Fin Ray Count

1.	29	21.	31	41.	32	61.	34
2.	29	22.	31	42.	32	62.	34
3.	29	23.	31	43.	32	63.	34
4.	29	24.	31	44.	32	64.	34
5.	29	25.	31	45.	32	65.	34
5.	29	25.	31	45.	32	65.	34
6.	29	26.	31	46.	32	66.	34
7.	29	27.	31	47.	32	67.	34
8.	29	28.	31	48.	33	68.	34
9.	29	29.	31	49.	33	69.	34
10.	29	30.	31	50.	33	70.	34
11.	31	31.	32	51.	33	71.	34
12.	31	32.	32	52	33	72.	34
13.	31	33.	32	53.	33	73.	35
14.	31	34.	32	54.	33	74.	35
15.	31	35.	32	55.	34	75.	35
16.	31	36.	32	56.	34	76.	35
17.	31	37.	32	57.	34	77.	35
18.	31	38.	32	58.	34	78.	35
19.	31	39.	32	59.	34	79.	35
20.	31	40.	32	60.	34	80.	35
						81.	35

29 — 10
31 — 20
32 — 17
33 — 7
34 — 18
35 — 9

			Total
N	= 81	500	581
EX	= 2,612	17,500	20,112
EX^2	= 84,494	612,500	696,994
$\frac{(EX)^2}{N}$	= 84,228.938	612,500	696,200.592
X	= 32.247	35.00	34.621

TABLE VI

(Continued)

$$S_b^2 = \frac{84{,}228.938 + 612{,}500.000 - 696{,}200.592}{1}$$

$$Sb^2 = 528.346$$

$$S_w^2 = \frac{84{,}494 + 612{,}500 - 696{,}728.938}{579}$$

$$S_w^2 = 0.458$$

$$F = \frac{528.346}{0.458} = 1160.4$$

Crit F ($_c$ 80 & 499 df) = 1.24

@ 95% level

TABLE VII

Summary of data on *Syngnathus scovelli* taken from Mississippi Sound side of Horn Island on 16 June 1964.

	TOTAL LENGTH IN MILLIMETERS	SEX	DORSAL FIN RAY COUNT	PECTORAL FIN RAY COUNT
1.	82	male +	31	16
2.	84	male +	32	16
3.	104	male +	29	14
4.	100	male —	32	16
5.	78	male +	34	16
6.	76	male +	32	16
7.	89	male +	29	16
8.	84	male 0	35	16
9.	84	male 0	32	16
10.	54	male —	34	16
11.	75	male +	35	14
12.	83	male 0	31	16
13.	76	male 0	34	16
14.	77	male +	32	16
15.	77	male +	32	16
16.	77	male +	34	16
17.	70	male 0	31	16
18.	68	male +	32	16
19.	85	male 0	33	16
20.	57	male 0	31	16
21.	56	male 0	29	16
22.	60	male 0	34	16
23.	82	male —	35	16
24.	66	male 0	31	16
25.	71	male +	31	16
26.	67	male 0	29	16
27.	66	male 0	32	16
28.	45	male 0	31	16
29.	74	male —	35	16
30.	81	male +	29	16
31.	71	male +	35	16
32.	67	male 0	32	16
33.	66	male 0	34	16
34.	45	male 0	29	16
35.	74	male —	31	16
36.	81	male +	34	16
37.	71	male +	33	16
38.	82	male —	31	16
39.	60	male 0	33	16

TABLE VII

(Continued)

	TOTAL LENGTH IN MILLIMETERS	SEX	DORSAL FIN RAY COUNT	PECTORAL FIN RAY COUNT
40.	65	male 0	32	16
41.	71	male —	34	16
42.	61	male 0	35	16
43.	65	male 0	32	16
44.	46	male 0	31	16
45.	46	male 0	31	16
46.	56	male 0	34	16
47.	50	male 0	32	16
48.	37	male 0	34	16
49.	41	male 0	32	16
50.	35	male 0	29	16
51.	52	male 0	31	16
52.	104	female	34	16
53.	106	female	34	16
54.	100	female	35	16
55.	97	female	31	16
56.	105	female	33	16
57.	116	female	35	16
58.	97	female	29	16
59.	86	female	31	14
60.	76	female	33	16
61.	95	female	34	16
62.	90	female	31	16
63.	45	female	32	16
64.	95	female	34	16
65.	85	female	32	16
66.	46	female	34	16
67.	73	female	31	16
68.	70	female	34	16
69.	65	female	31	16
70.	58	female	32	16
71.	64	female	29	16
72.	42	female	31	16
73.	36	female	34	16
74.	40	female	35	16
75.	48	female	29	16
76.	64	female	31	16
77.	61	female	33	16
78.	62	female	34	16
79.	59	female	31	16
80.	71	female	33	16
81.	42	female	32	16

\+ with eggs in pouch

— empty brood pouch

0 no evident brood pouch

recent population which is not renewed each spring by flood waters from the Tensas River (Plate III, Fig. 1). If this were a very old population, or if it were renewed each spring, more variation should be evident. This population may have been established any time from the present to about 1,000 years ago when the Mississippi River channel crossed the present lake site.

So far as is known, this is the only species of fish in North America known to have breeding populations in both fresh and salt water, with the possible exception of *Mollienesia latipinna* which breeds in fresh and brackish waters. In view of Gunter's (1942) remarks concerning the distribution of *S. scovelli* over the Peninsula of Florida, the discovery of a breeding population in Lake St. John suggests that breeding populations may be present in the fresh waters of Florida which have not been detected as yet.

Editor's Note:

After this article was in page proof, my attention was called to W. M. McLane, (The fishes of the St. John's River System. Doctoral Thesis, University of Florida 1955. Typescript pp. 5 + 361.) in which the author records breeding Syngnathus scovelli *in the fresh waters of Florida. There are several interesting comparisons between the data of McLane and Whatley, but they cannot be treated here.—G. G.*

The fact that breeding of this pipefish is now known to take place in both fresh and salt water raises some interesting questions concerned with osmoregulation. Even so it should be noted that osmotic problems of the developing eggs are minimized by the fact that transfer of eggs from the female to the male is extremely rapid and the eggs are carried within the brood pouch, where presumably they are maintained in an optimum osmotic environment. This rapid transfer of eggs from the female to the male with a very brief exposure to the surrounding water would seem to explain in part the ability of this fish to breed in both fresh and sea water. In *Mollienesia latipinna* fertilization is internal and the eggs are never exposed.

It is possible, though difficult, to maintain *S. scovelli* in fresh water aquaria, chiefly because a readily available supply of live plankton is essential to their maintenance. Optimum temperature appears to range between 72 F and 80 F. Aquaria should be placed in a shady spot with aquatic vegetation, preferably naiad *(Najas guadalupensis)* placed in the tank.

The metabolic rate of *S. scovelli* is apparently high and a ready supply of plankton permits a very rapid growth of the young from 12 mm up to 80 mm in length. From 27 June 1964 until 7 July 1954, young pipefish maintained in plastic buckets of sea water at the Gulf Coast Research Laboratory increased in length from 12 mm at birth to 28 mm in less than two weeks. From 27 June 1964 until September 1964, three specimens increased their length from 12 mm to 60 mm. This represents a 5-fold increase in length in a period of a little more than three months. Growth slows down at around 80 mm length in both salt water and fresh water forms.

S. scovelli apparently has a life cycle of approximately three years.

There is an apparent difference of trunk to tail ratio in mature males when compared with mature females (Plate IV, Graph 1). Males have comparatively shorter trunks and longer tails with reverse condition being present in females. These differences are not as apparent in juvenile forms.

S. scovelli is gregarious in nature and is normally found in vegetation on the bottom in cooler, more shaded areas. Males with eggs in their brood pouches tend to become solitary or to form groups with other egg-bearing males.

S. scovelli becomes quite seahorse-like in its appearance during mating and both females and males shake their heads violently back and forth in the course of this activity. The actual oviposition is extremely rapid. The incubation period is dependent upon temperature and may vary fram 12 to 18 or more days.

This is the first record in the literature of the breeding behavior of *S. scovelli*. The female is the more aggressive partner in the initial breeding behavior and will oviposit in the male's brood pouch soon after one brood is born. A favorable temperature could mean that the male could incubate 20 or more clusters of eggs yearly in his brood pouch dependent upon an incubation period of 12 to 18 days each time.

Some *S. scovelli* males practiced cannibalism upon their young when kept in containers in the laboratory. This situation may or may not be duplicated in natural conditions.

The range in size of specimens taken from Lake St. John was 18 mm to 160 mm.

Fresh water *S. scovelli* appear to be more robust than their marine counterparts. Marine specimens from Davis Bayou and Horn Island range up to 116 mm in my collection. Fresh water specimens range to 160 mm. Many marine male specimens with eggs in their brood pouches range from 71 mm to 104 mm (Table VII). Fresh water males taken with eggs in their brood pouches ranged from 120 mm to 160 mm.

The answers to the questions posed in the introduction appear to be: (1) this fish is representative of the population designated as *S. scovelli*, (2) this population is strikingly homogenous, (3) a similar population exists in Lake Bruin, Louisiana, (4) this fish can be maintained in fresh water aquaria, (5) this population was probably established in Lake St. John sometime during a period 1,000 years ago until as recently as the 1927 flood, (6) this fish has a life span of approximately three years. The initial rate of growth of *S. scovelli* is extremely rapid.

LITERATURE CITED

Breder, C. M., Jr. 1948. Field book of marine fishes of the Atlantic coast. G. P. Putnam's Sons. New York and London. 332 p.

Eddy, S. 1957. How to know the freshwater fishes. W. C. Brown Co. Dubuque, Iowa. 253 p.

Evermann, B. W. and W. C. Kendall. 1894. The fishes of Texas and the Rio Grande Basin, considered chiefly with reference to their geographic distribution. Bull. U. S. Fish. Comm. 12:57-126.

Evermann, B. W. and W. C. Kendall 1896. Description of a new species of pipefish *(Siphostoma scovelli)* from Corpus Christi, Texas. Proc. U. S. Nat. Mus. 18:113-115.

Evermann, B. W. and W. C. Marsh. 1902. The fishes of Puerto Rico. Bull. U. S. Fish. Comm. 20:51-350.

Fernald, M. L. 1950. Gray's Manual of Botany. 8th Ed. Amer. Book Co. New York. 1632 p.

Gunter, G. 1938. Notes on invasion of fresh waters by fishes of the Gulf of Mexico with special references to the Mississippi-Atchafalaya River System. Copeia 2:69-72.

Gunter, G. 1942. A list of the fishes of the mainland of North and Middle America recorded from fresh water and sea water. Amer. Midl. Nat. 28(2):305-326.

Gunter, G. 1945. Studies on marine fishes of Texas. Publ. of the Inst. of Marine Sci. U. of Texas. 1:1-190.

Gunter, G. 1952. Historic changes in the Mississippi River system. Publ. of the Inst. of Marine Sci. U. of Texas. 2:118-138.

Gunter, G. 1956. A revised list of euryhalin fishes of North and Middle America. Amer. Midl. Nat. 56(2):345-354.

Herald, E. S. 1941. A systematic analysis of variation in the Western American pipefish *Syngnathus californiensis*. Stanford Ichthy. Bull. Stanford U. 2(3):49-73.

Herald, E. S. 1942. Three new pipefishes from the Atlantic coast of North and South America, with a key to the Atlantic American species. Stanford Ichthy. Bull. Stanford U. 2(4):125-134.

Herald, E. S. 1961. Living fishes of the world. Doubleday and Co., Inc. Garden City, New York. 304 p.

Herre, A. W. 1927. Gobies of the Philippines and the China Sea. Mongr. Bur. Sci. Philippines Isl. 23(1):1-352.

Hutchinson, G. E. 1957. A treatise on limnology, geology, physics, and chemistry. John Wiley and Sons, Inc. New York. 1015 p.

Jordan, D. S. and B. W. Evermann. 1896. The fishes of North and Middle America. Bull. U. S. Nat. Mus. 47:761-762.

Lambou, V. W. 1961. Fish population of Mississippi River oxbow lakes in Louisiana. Proc. La. Acad. of Sci. 23:52-64.

Linnaeus, C. 1758. Systema Naturae. 10th Ed. 1:1-336.

Muenscher, W. C. 1944. Aquatic plants of the United States. Comstock Publ. Co., Inc. Cornell U. 374 p.

Myers, G. S. 1964. A brief sketch of the history of ichthyology in America to the year 1850. Copeia 1:33-41.

Reid, G. K., Jr. 1954. An ecological study of the Gulf of Mexico fishes in the vicinity of Cedar Key, Florida. Bull. Marine Sci. Gulf and Caribbean. 4(1):1-94.

Renfro, W. C. 1960. Salinity relations of the fishes in the Arkansas River, Texas. Tulane Studies. Zool. 8(3):83-91.

Richmond, E. Avery. 1962. The fauna and flora of Horn Island, Mississippi. Gulf Research Reports. 1(2):59-106.

Simmons, Ernest G. 1957. An ecological survey of the upper Laguna Madre. Publ. Inst. Marine Sci. U. of Texas. 4(2):156-200.

Springer, V. G. and K. D. Woodburn. 1960. An ecological study of fishes of the Tampa Bay area. Prof. Papers Fla. State Board of Cons. Marine Lab. St. Petersburg, Florida. 1:1-104.

Steel, R. G. D. and J. H. Torrie. 1960 Principles and procedures of statistics. McGraw-Hill Book Co., Inc. New York. 481 p.

Whatley, E. C. 1962. Occurrence of breeding Gulf pipefish, *Syngnathus scovelli,* in the inland fresh waters of Louisiana. Copeia 1:220.

Witt, A., Jr. and R. S. Campbell. 1959. Refinements of equipment and procedures in electro-fishing. Trans. Amer. Fish. Soc. 88(1): 33-35.